The Journal of Laboratory and Clinical Medicine

VOL. VII ST. LOUIS, MARCH, 1922 NO. 6

ORIGINAL ARTICLES

AN EXPERIMENTAL INVESTIGATION OF THE PHARMACOLOGICAL ACTION OF QUINIDINE*

BY D. E. JACKSON, PH.D., M.D., ALFRED FRIEDLANDER, M.D., AND J. V. LAWRENCE, B.S.

WE HAVE recently heard a well-known pharmacologist make the suggestion that a now very extensively used medicinal compound which has been in the hands of the medical profession for only a few years was introduced into therapeutics at a time when its general toxic action, the size of its fatal dose and its specific action on the various vital organs and functions of the body were almost entirely unknown. These things have since been very largely determined—mainly by trial on the human species. And the same investigator has further suggested that in the alkaloid quinidine we have a drug whose general toxicology, and whose specific actions on the heart, respiratory center and other vital organs should be determined, in so far as may be possible, by animal experimentation before the necessity is at hand of accumulating data on such points as these by the results of disastrous experiments on the human subject.

Quinidine is the dextrorotary isomer of quinine. The melting point of quinidine is 168-171.5°. It is soluble in ether and alcohol, less so in chloroform and benzol and but slightly soluble in water. The sulphate is the usual commercial salt and the common impurities consist generally of traces of hydroquinine and hydroquinidine. If 0.2 gram of quinidine be added to 2 c.c. of concentrated sulphuric acid and then 0.5 c.c. of hydrogen peroxide be added, a yellow color will be produced. This gradually turns to deep orange and finally fades out entirely.

In the present work we have used different samples of the sulphate as prepared by Powers, Weightman and Rosengarten, and also a sample of the

*From the Departments of Pharmacology and Internal Medicine of the University of Cincinnati College of Medicine, Cincinnati, Ohio.

pure alkaloid as obtained fresh from C. A. F. Kahlbaum. The latter preparation was dissolved by the addition of a small amount of acid. All samples have possessed identical actions on the body so far as we were able to determine.

The recently discovered action of quinidine on auricular fibrillation and its promise of extensive clinical utilization is a therapeutic finding of the first importance. Its miraculous effects in some cases and its apparently fatal action in a certain number of other cases renders a thorough, comprehen-

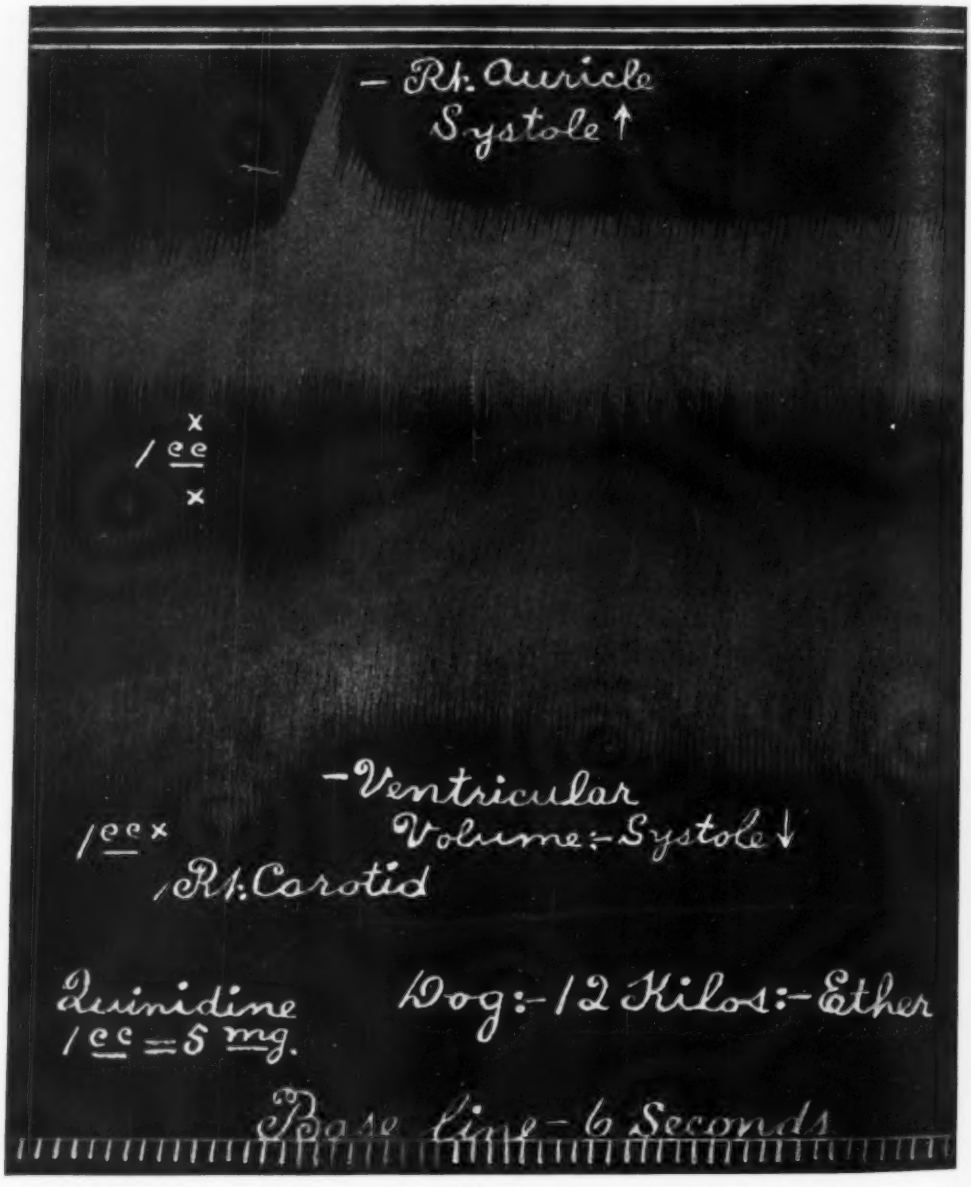

Fig. 1.

sive and accurate study of its actions in the body exceedingly desirable. This will of necessity require a long time. From the clinical papers appearing to date, it would seem that no key to the determination of what cases should, or should not, be exposed to quinidine treatment has yet been found. The best suggestion has simply been the advice to employ the drug with caution, beginning with one or two small doses (0.2 gram) to test the patient for idiosyncrasies, to use the drug only in well ordered hospitals and to keep the patient under constant, careful observation preferably by means of the string galvanometer or polygraph, records being made at short intervals while the drug is being administered. Notwithstanding all precautions it seems that a few unfortunate, and even fatal results, have been brought about, in a few instances after a normal sinus rhythm had been established, and the patient had appeared to be making entirely satisfactory progress. Such cases render

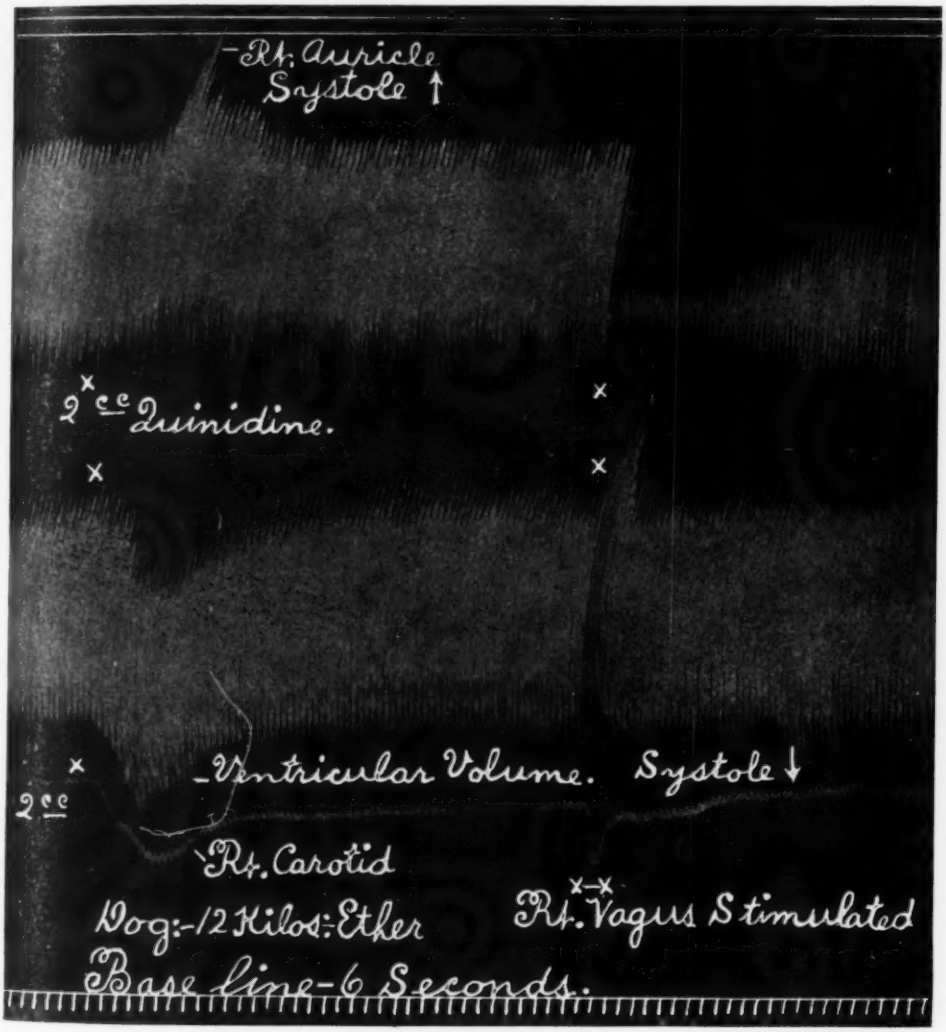

Fig. 2.

a more complete knowledge of the action of the drug, and the indications for its use imperative.

In the present work we have primarily studied the action of quinidine on the normal animal, but we have varied the conditions and the nature of the experiments over an extensive field for the purpose of gaining information regarding the action of the drug from as many different sources and angles as possible.

We can perhaps best describe our observations by reference to the tracings we have selected for inclusion in this article. Fig. 1 was made from a dog with the chest open. A system of levers connected by means of a thread passing over two pulleys was used to record the action of the right auricle, while the ventricular volume was recorded by means of an ordinary cardi-

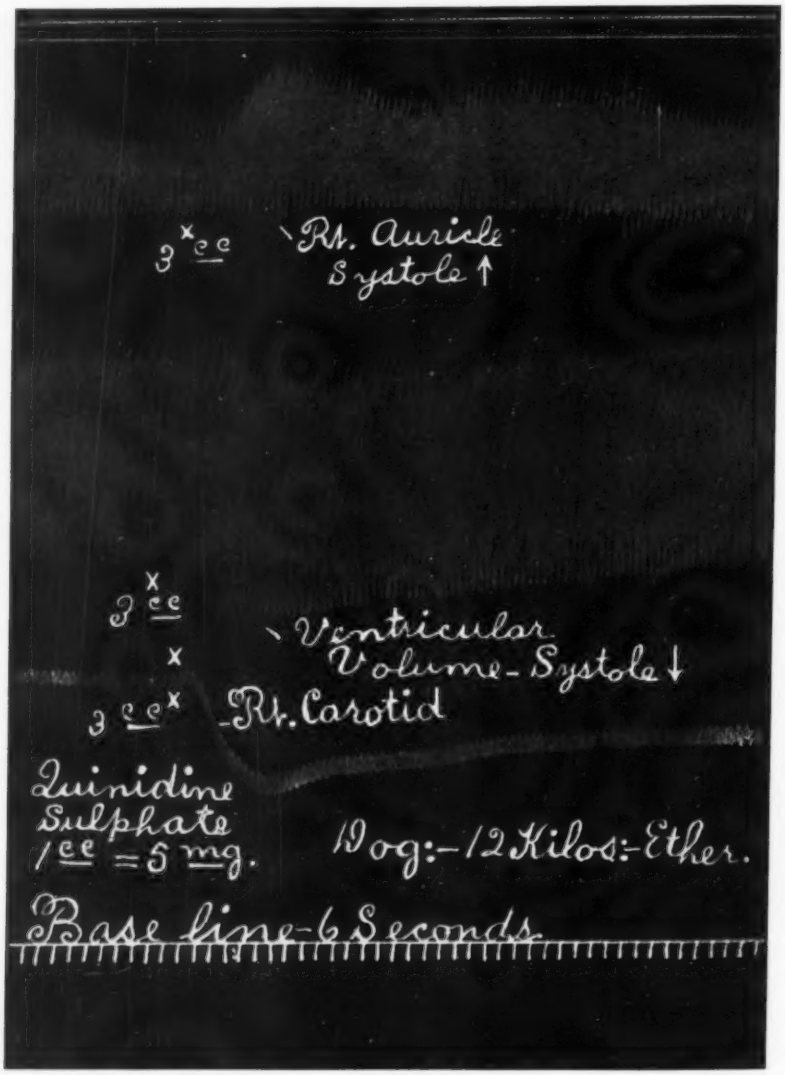

Fig. 3.

ometer connected to a recording tambour. Blood pressure was taken with a mercury manometer. At the point indicated by the cross, 1 c.c. (5 mg.) of quinidine sulphate solution was injected by way of the femoral vein. Contrary to what might be expected, the auricular rate was accelerated and the extent of the systole appeared to be somewhat increased. The ventricle showed a dilatation, but the extent of diastole and systole were but slightly affected, if indeed they were changed at all. The blood pressure fell, and this is the most characteristic and typical action of the drug when injected intravenously.

Fig. 2 (which was made from the same animal following Fig. 1) shows the result of injecting 2 c.c. (10 mg.) of quinidine. Again the auricle is apparently stimulated while the blood pressure falls. The shrinkage in total volume of the ventricle at first is probably due to the general fall in systemic arterial pressure, rather than to a direct action on the heart. As the pressure slowly rises, the heart volume comes back to normal and perhaps a little beyond; i.e., a slight further ventricular dilatation occurs. Thus far the animal had received 15 mg. of the drug. The right vagus nerve was then isolated in the neck and stimulated. At once a marked inhibition of the heart was produced. We suspect the inhibition of the auricle was a little more marked, and a little more prolonged than would have occurred if the quinidine had not been given.

Fig. 3 was made still later in the experiment. At this time the auricular beat was feeble, but the blood pressure remained fairly high. An injection of 3 c.c. (15 mg.) was given and a marked increase in both rate and amplitude of the auricular contractions was seen at once. In fact, the auricular tracing bears a striking resemblance to one produced in a weak heart by a small dose of epinephrin. From most published work one would not expect to find such action on the auricle. The systemic pressure is here again lowered by the injection while the volume of the ventricle shrinks. The extent of its filling and emptying, however, is but little, if any, affected.

Fig. 4 is a tracing made from a different animal. In this case, the auricular record was made by a lever as in Fig. 1, but the ventricular record in Fig. 4 was made by means of a myocardiograph, which records primarily only the systole and diastole of the ventricle. Here it is seen that an injection of 2 c.c. (10 mg.) again stimulates the auricle. And even the ventricular tracing indicates stimulation rather than depression. The typical fall in blood pressure is again produced. It is probable that part of the alteration in rate and amplitude of the heart chambers, as indicated by the tracings, is due secondarily to the fall in blood pressure. This would tend to allow the heart to beat faster, and the increased speed would cause the very light writing levers to manifest a certain amount of fling as a result of the momentum which they acquire in their upward and downward movements. But this cannot account for all of the change which the drug produces in the heart chambers.

Further evidence on this point was accordingly sought by giving quinidine to an animal which had previously received a certain amount of aconitine. This drug possesses a striking action on the heart, and Fig. 5 shows the

peculiar weakening and irregularity produced in the auricle by the drug. The ventricular record was made by means of a myocardiograph. After the auricular record had become very weak and irregular, a dose of 2 c.c. (10 mg.) of quinidine was injected. A prompt increase in amplitude and regularity was produced in the auricle. The ventricular changes were less marked. But in this case the heart muscle must have been in a very peculiar condition as a result of the aconitine action. This drug renders the heart muscle more irritable and with large doses produces great irregularity and arrhythmicity. In the earlier stages the heart is slowed because of a strong medullary stimulation of the cardioinhibitory center by the drug. It has been found that after section of the vagi, or after atropine, a much larger dose of aconitine is required to produce irregularity of the heart than is necessary in

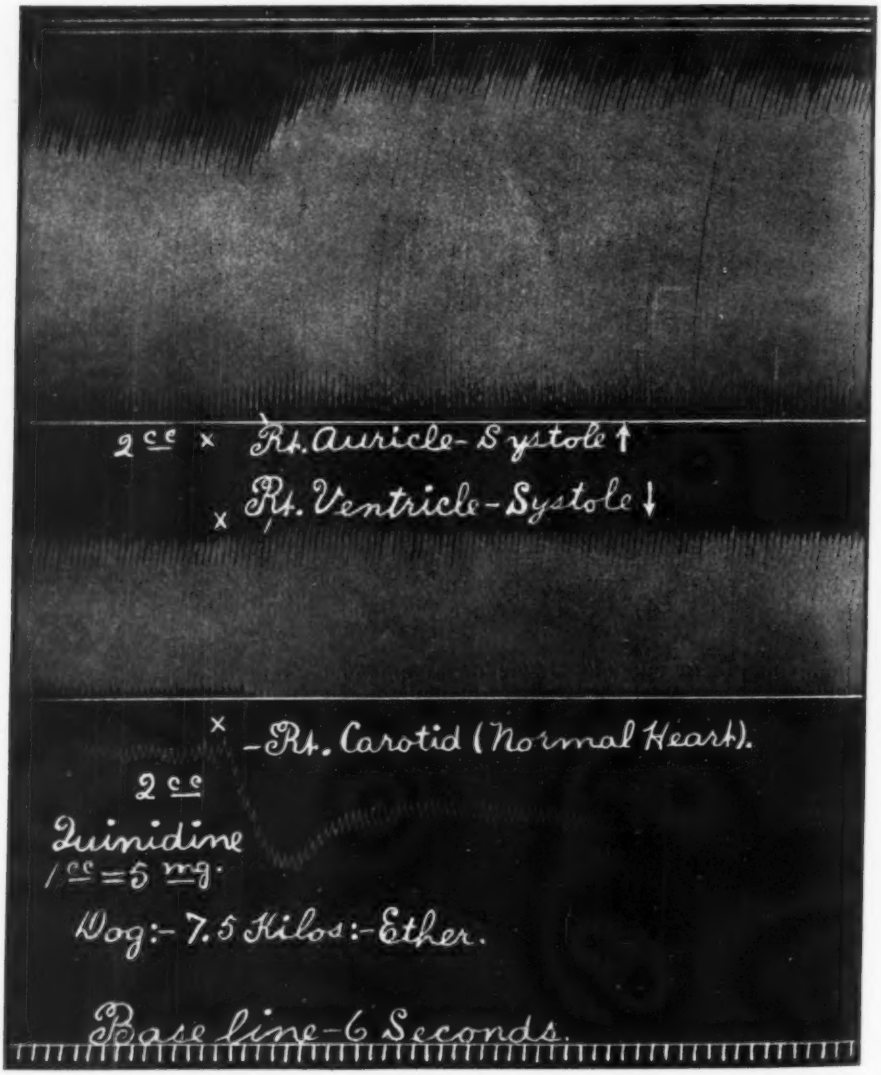
2 c.c. × Rt. Auricle - Systole ↑
× Rt. Ventricle - Systole ↓
× - Rt. Carotid (Normal Heart).
2 c.c.
Quinidine
1 c.c. = 5 mg.
Dog:- 7.5 Kilos:- Ether.
Base line - 6 Seconds.

Fig. 4.

a normal animal. We strongly suspect that quinidine in some way must affect the same mechanism in the heart which is involved in this last peculiar action of aconitine. In Fig. 5 it is seen that quinidine actively counteracts the irregularity which had been set up by the aconitine. It is conceivable that if, in this animal, that influence which section of the vagi interposes to hinder or delay development of cardiac arrhythmia had been suddenly increased just at the time the quinidine was injected, then that influence might have restored the heart again to a regular rhythm. In Fig. 6 the action of quini-

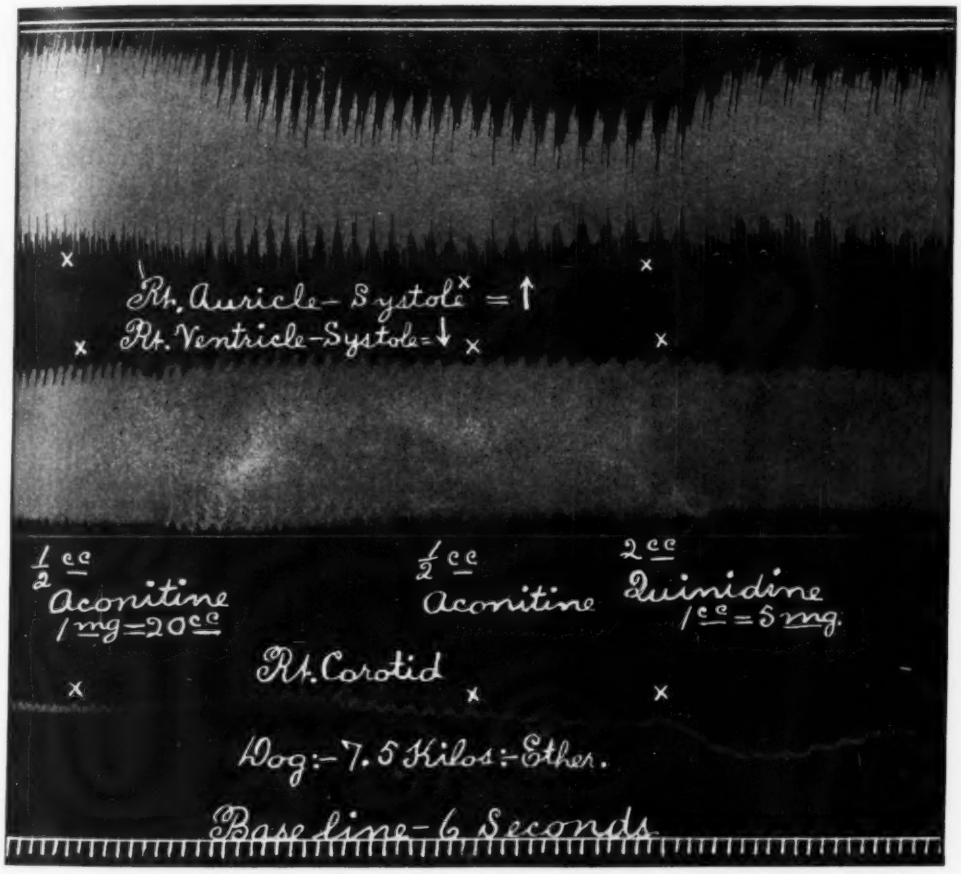
Rt. Auricle-Systole = ↑
Rt. Ventricle-Systole = ↓
½ cc Aconitine 1 mg = 20 cc
½ cc Aconitine
2 cc Quinidine 1 cc = 5 mg.
Rt. Carotid
Dog:- 7.5 Kilos:- Ether.
Baseline - 6 Seconds

Fig. 5.

dine on auricular irregularity is again shown in a striking manner. The blood pressure tracing here, however, shows that the effectiveness of the heart is not much, if any, improved by the action of quinidine on the auricle. But the stage of aconitine intoxication in which the animal happens to be at the time the quinidine is given has a great deal to do with the results obtained. We have investigated this point by injecting quinidine very early in the stage of aconitine poisoning, when the heart had just developed a marked (and probably lasting) irregularity. Then in other animals the aconitine action has been developed further and further before the initial dose of

quinidine has been given. The following tracings will illustrate clearly the opposing effects which aconitine and quinidine have on the heart.

Fig. 7 shows the earliest development of cardiac irregularity just after a dose of ¼ c.c. of the aconitine solution had been injected. More aconitine had been injected previously in very small, repeated doses as it was desired to bring on cardiac irregularities very gradually. Some central vagus stimulation was present here as indicated by the gradual fall in pressure in the beginning of the tracing. The blood pressure (mercury manometer) shows fairly well the action going on in the heart. The irregularity which suddenly appears after the aconitine is injected, therefore, is influenced by both the central inhibitory action and the direct muscular stimulation of the aconitine. It is probable that an irregularity of this particular type would not cease abruptly and automatically without some special influence being brought to bear on the heart. Consequently it is fair to presume that the injection of 1 c.c. (5 mg.) of quinidine caused the heart to return to an approximately

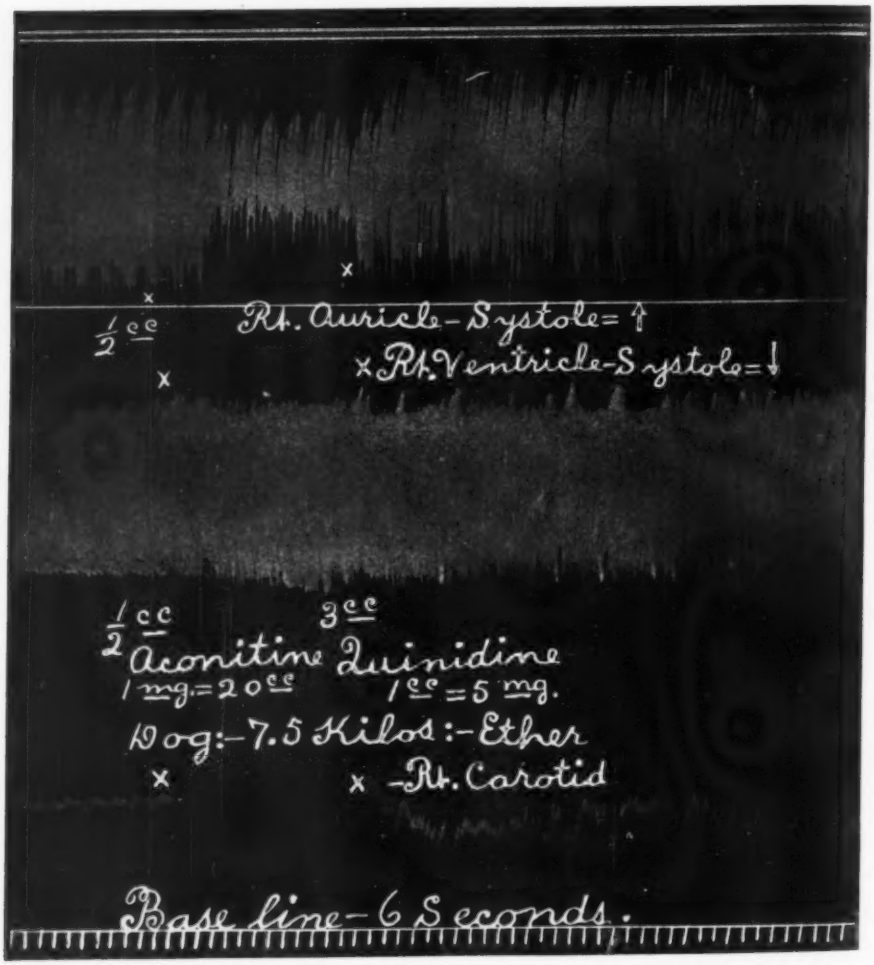

Fig. 6.

normal rhythm. After the initial fall the pressure again rose to approximately its normal level and the respiration again improved, probably partly because of the improved circulation. It can be noted, however, that after a time the effects of the quinidine tend to wear off and the aconite action reappears

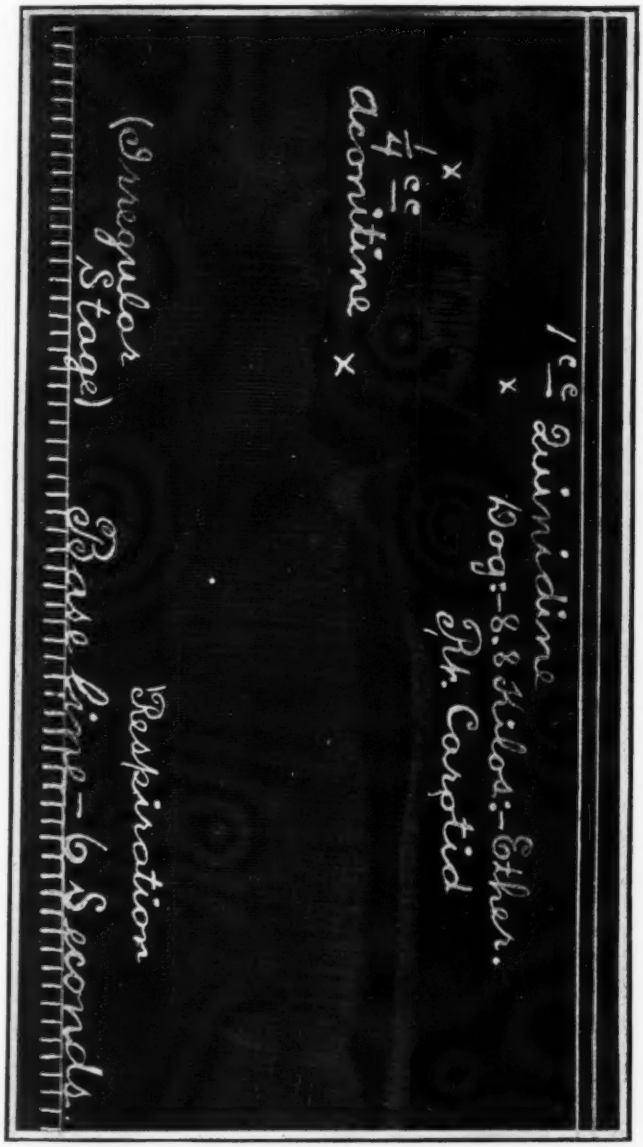

Fig. 7.

even though no further injections of the drug are made after the quinidine is given. This temporary character of the quinidine action is perhaps similar to that observed over periods of a few hours, or a few days in clinical cases in which fibrillation of the auricles is checked temporarily, but in which a recurrence of the fibrillation soon occurs.

Fig. 8 shows a more marked development of aconitine irregularity before

the quinidine is injected. In this case, following $\frac{1}{10}$ c.c. of aconitine solution, there is developed a very marked central vagus stimulation as shown by the fall in pressure and the long, slow heart beats. The cardiac muscle is also

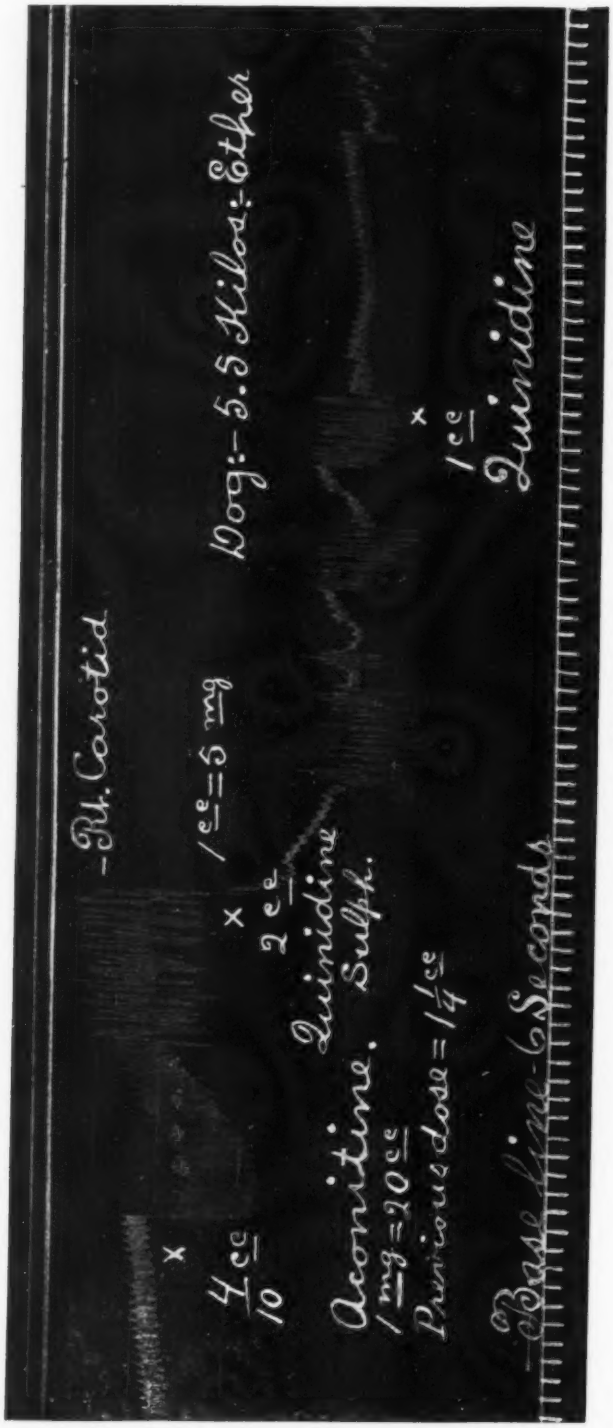

Fig. 8.

becoming highly irritable as is shown a little later by the sudden irregular appearance of extrasystoles, missed ventricular beats, and perhaps other types of arrhythmia. It is certain that the heart here would not spontaneously have returned to a normal rhythm. Therefore, 2 c.c. (10 mg.) of quinidine

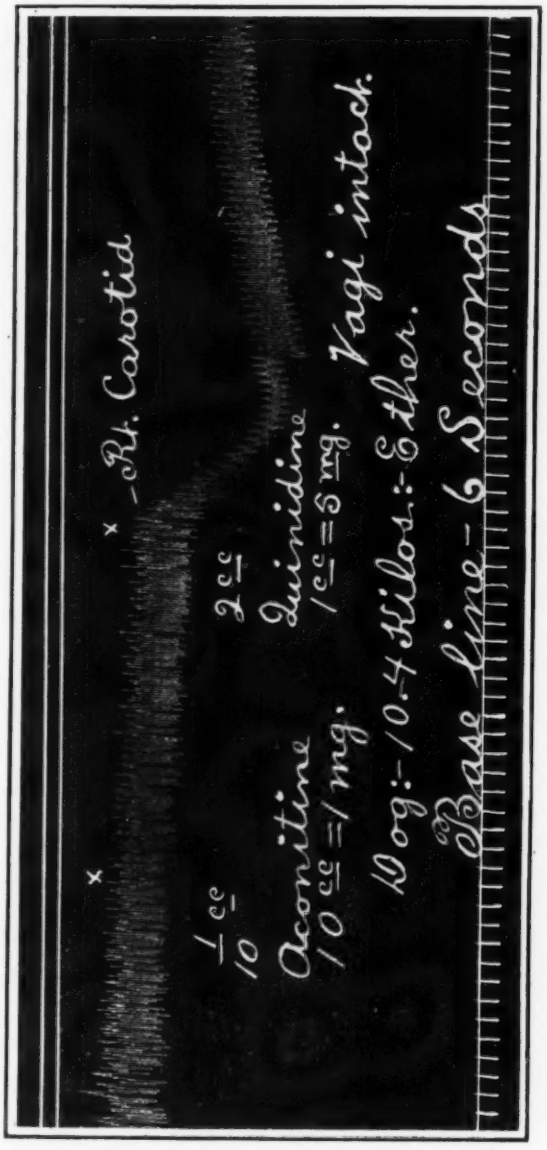

Fig. 9.

were injected at the point indicated. A prompt fall in pressure followed, but for a short time the heart was restored to a normal rhythm. The aconite, however, continued to act and in a short time the previous arrhythmia again started up. This continued intermittently for a time, and at the point indicated a second injection (1 c.c., 5 mg.) of quinidine was given. This time a completely normal sinus rhythm was produced and continued for more than

a minute, after which the aconite action again began to manifest itself. This tracing shows quite well the counteracting action which quinidine possesses against poisonous doses of aconite, if the quinidine be injected fairly early in the course of the intoxication.

Fig. 9 shows this same point, but here the action of the aconite (repeated doses) had progressed still farther before the quinidine was given than was the case in Fig. 8. The strong vagus inhibition is shown in the latter part

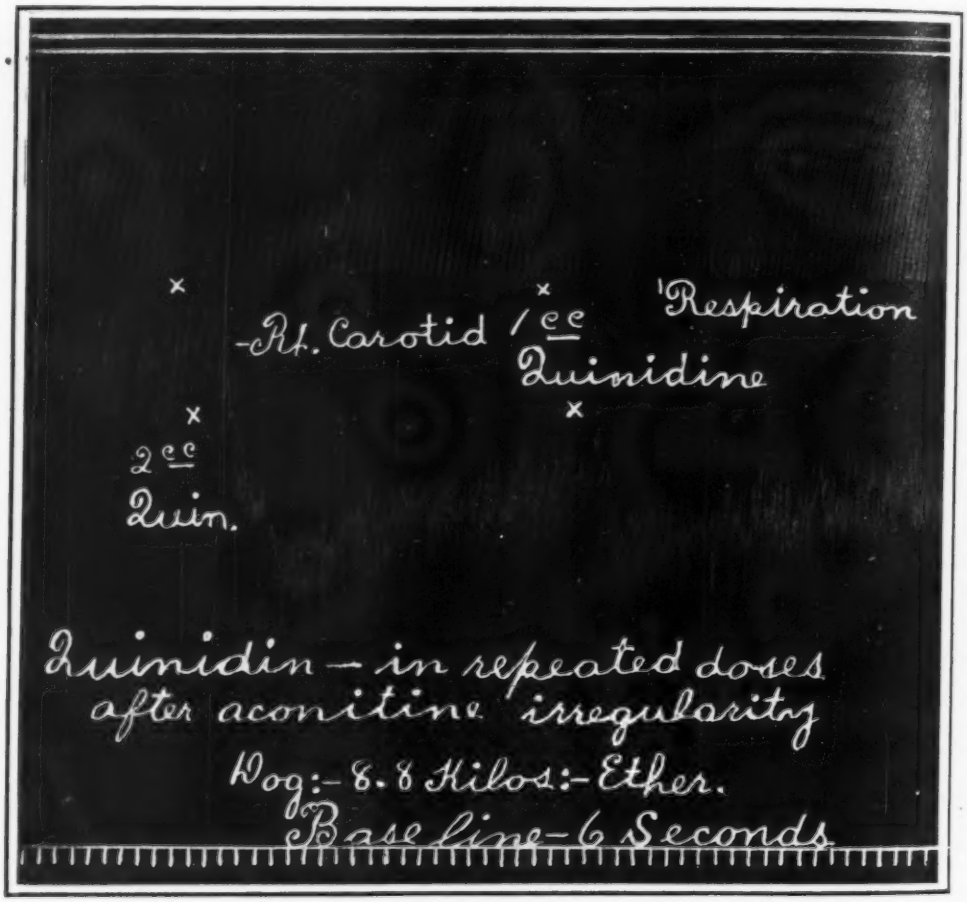

Fig. 10.

of the tracing as it slowed the heart and checked the regular sinus rhythm which the quinidine had temporarily established.

Fig. 10 (which was made from the same dog as that used to produce Fig. 7) shows the complete failure of quinidine to overcome the cardiac irregularity which is established in the late stages of aconitine poisoning. This point is of some clinical interest because of the failure of quinidine to stop auricular fibrillation in a certain number of cases. These cases cannot now be known beforehand because there are no known signs or symptoms to serve as a basis for selection. But the fact that quinidine will stop auricular

fibrillation in about 50 per cent of all clinical cases, while in the remaining 50 per cent some cases can be changed from a fibrillation to a flutter, others show no change, while in still others an acceleration may apparently occur,

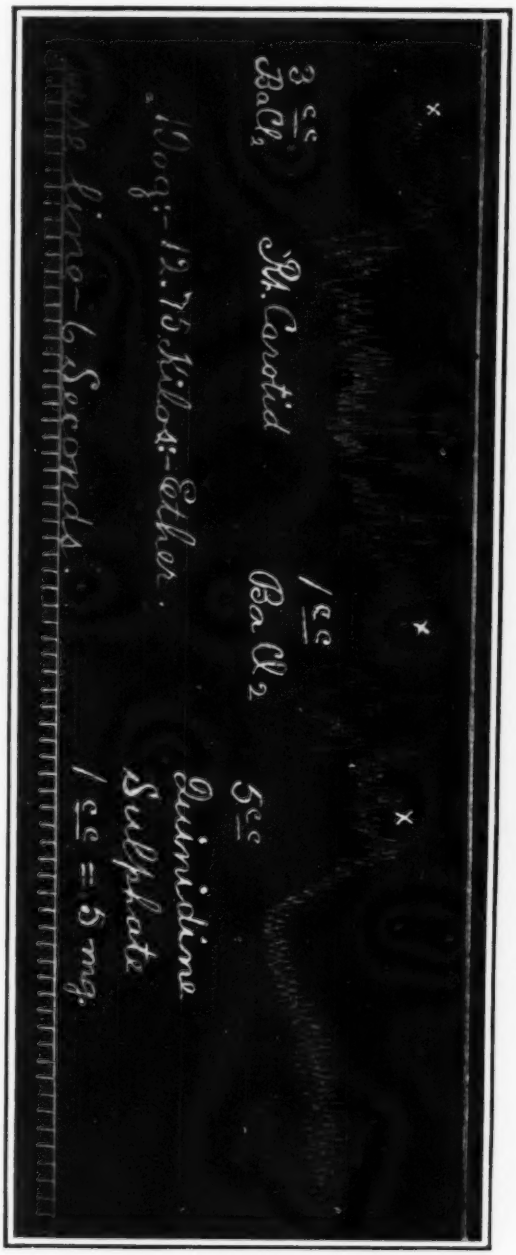

Fig. 11.

indicates that there are probably at least two types of auricular fibrillation. It is to be noted, of course, that aconite sets up irregularities in both auricles and ventricles and that these irregularities may not be analogous to those occurring clinically as auricular fibrillation or auricular flutter. On the other

hand, we have selected aconite, digitoxin and barium to set up cardiac arrhythmias because these drugs come more nearly to producing such cardiac irregularities as probably occur clinically than any other drugs with which we are acquainted. And furthermore these drugs involve both the nervous mechanisms controlling the heart, and the heart muscle itself in their actions. Consequently, it was hoped that a study of the action of quinidine on the irregularities set up by these drugs might throw some light on the action of quinidine itself.

Fig. 11 shows the development of a marked (muscular) irregularity in

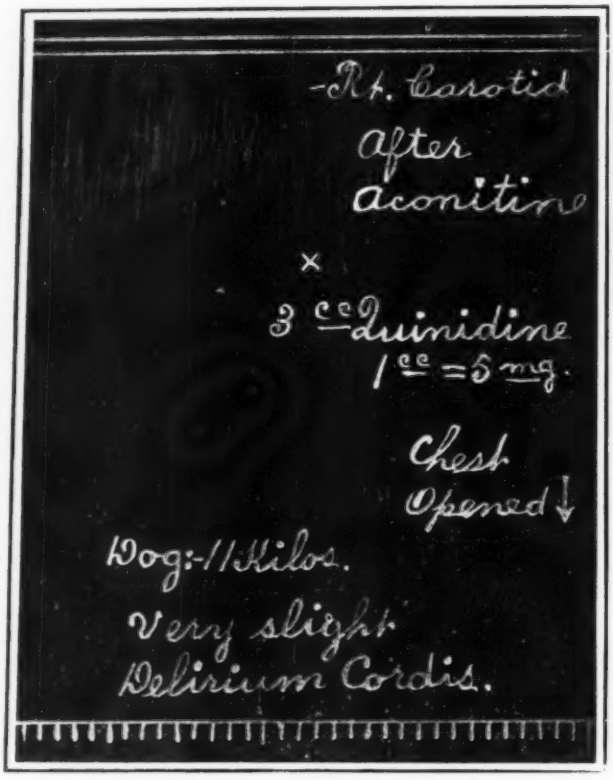

Fig. 12.

the heart as the result of repeated injections of barium chloride. The tracing was made with a mercury manometer. Presumably these irregularities are of purely muscular origin. It will be seen that an injection of 5 c.c. (25 mg.) of quinidine produced a distinct regulatory effect on the cardiac mechanism. While this is less marked, perhaps, than is that following quinidine in the early stages of aconite irregularity, it is still fairly evident and is of interest because here quinidine is counteracting a presumably purely muscular type of irregularity. In the later stages of barium irregularity quinidine is also unable to restore a normal rhythm. Clinically it has been observed that cases of auricular fibrillation of long standing appear to be much less amenable to quinidine treatment than are recent cases.

Cases in which death occurred after quinidine, apparently rather suddenly in some instances, have prompted us to include here Figs. 12 and 13. In Fig. 12 the animal had been given aconitine until a marked irregularity of the heart appeared. The pressure was high, and the possibility, of course, existed that delirium cordis might occur at any time. We then injected 3 c.c. (15 mg.) of quinidine solution. Almost at once the heart was stopped, presumably by the quinidine. We hurriedly opened the chest and found only a very slight delirium cordis to be present. We have repeatedly seen this counteracting action of quinidine, which either partially or entirely prevents the development of a delirium cordis after such drugs as aconite or digitoxin. Fig. 13 shows an abrupt fatal termination caused by an injection of quinidine following marked cardiac irregularity (3rd stage) produced by digitoxin.

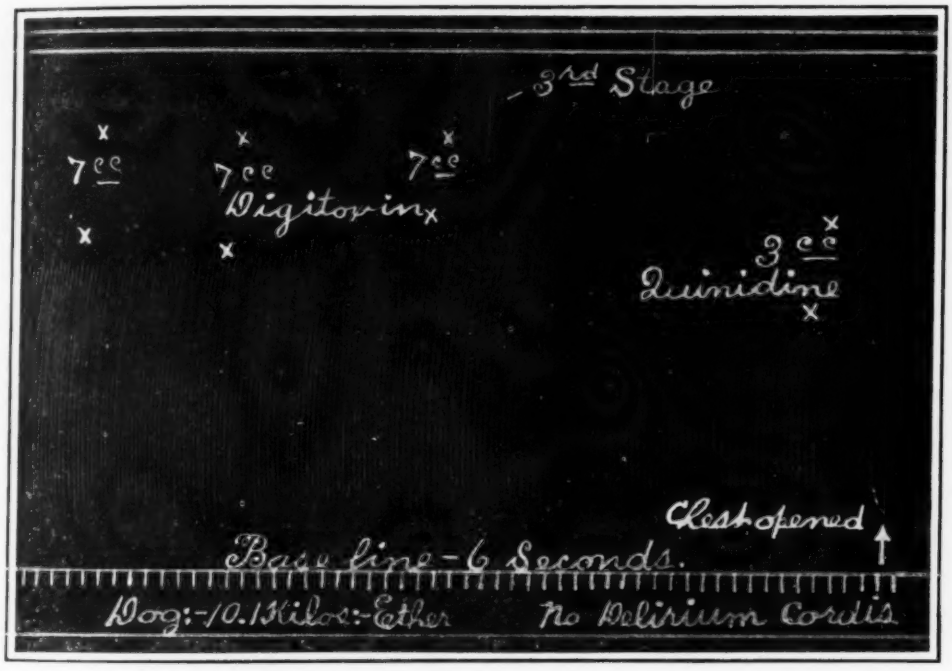

Fig. 13.

The chest was opened immediately and no delirium cordis was found as would have been expected from the action of the digitoxin.

Figs. 14 and 15 represent another type of experiment in which cardiac irregularities were established by a different method. In this case the chest was opened and the heart exposed. With a fine pointed hypodermic syringe an injection of a few minims of a mixture consisting of equal parts of alcohol and chloroform was made into the base of the ventricle at the position of the auriculoventricular junction. The object was to establish heart block by injury to the bundle of His. In this type of experiment it is possible to produce auricular fibrillation or flutter by direct Faradization of the auricles. As will be seen from the tracings marked irregularity of the heart was

produced. We assume that this irregularity may bear some resemblance to that occurring clinically in auricular fibrillation or flutter. In both tracings it can be seen that quinidine exercised a distinct regulatory effect on the heart beat. And this effect appears to be rather more lasting in its character than is that which usually follows irregularities produced by aconite, digitoxin or barium.

Fig. 16 is presented for the purpose of demonstrating one point. It has

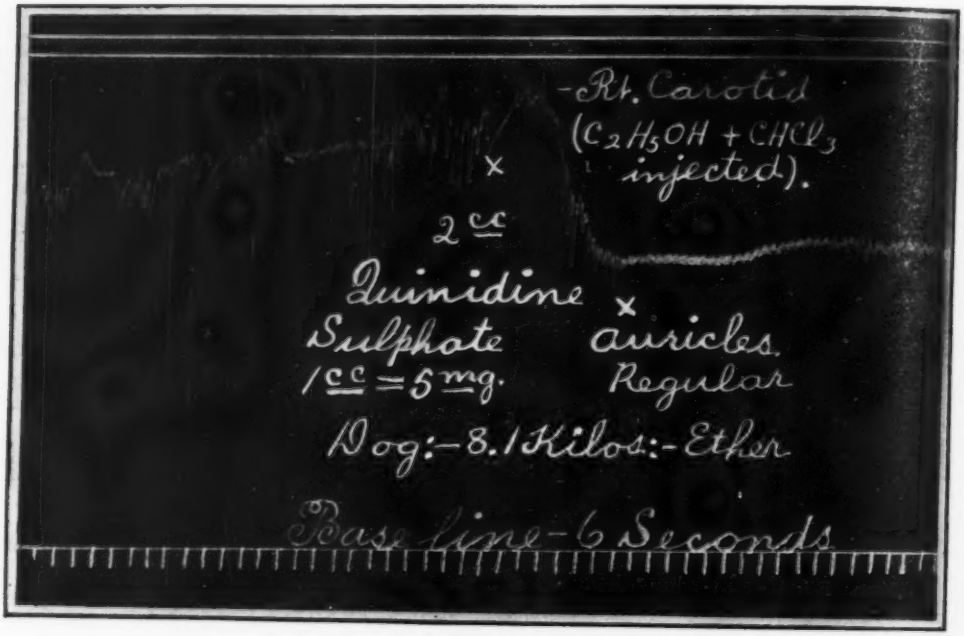

Fig. 14.

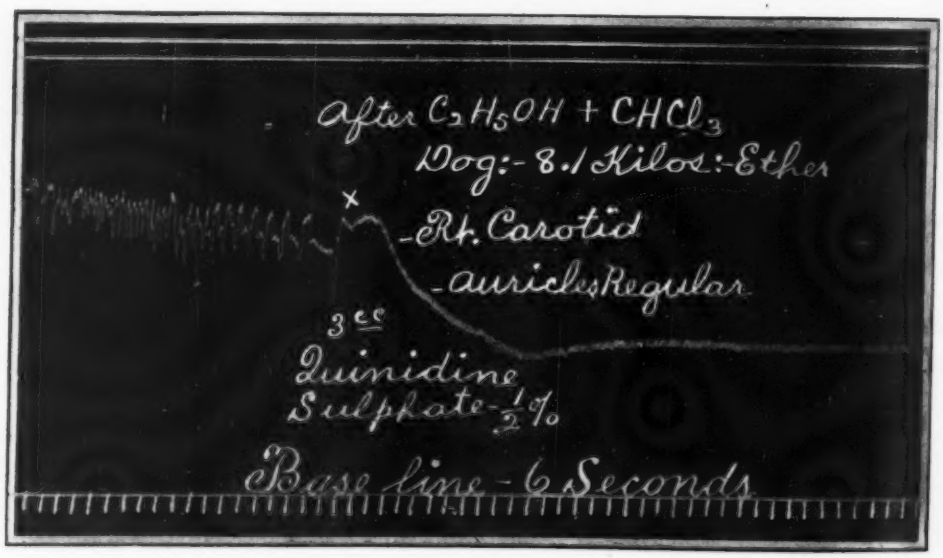

Fig. 15.

been supposed that quinidine paralyzes the inhibitory endings of the vagi in the heart. This action has been described as being similar to, but milder than, that of atropine. In Fig. 16 the records of the auricular and ventricular contractions are shown. The animal had previously been given 100 milligrams of quinidine in small repeated doses. At the time when this tracing was made the blood pressure had been reduced to a very low level by the quinidine and we feared to give another dose of the drug because it would probably stop the heart (the animal was getting artificial respiration). Our object was to give the quinidine all possible opportunity to act on the vagi

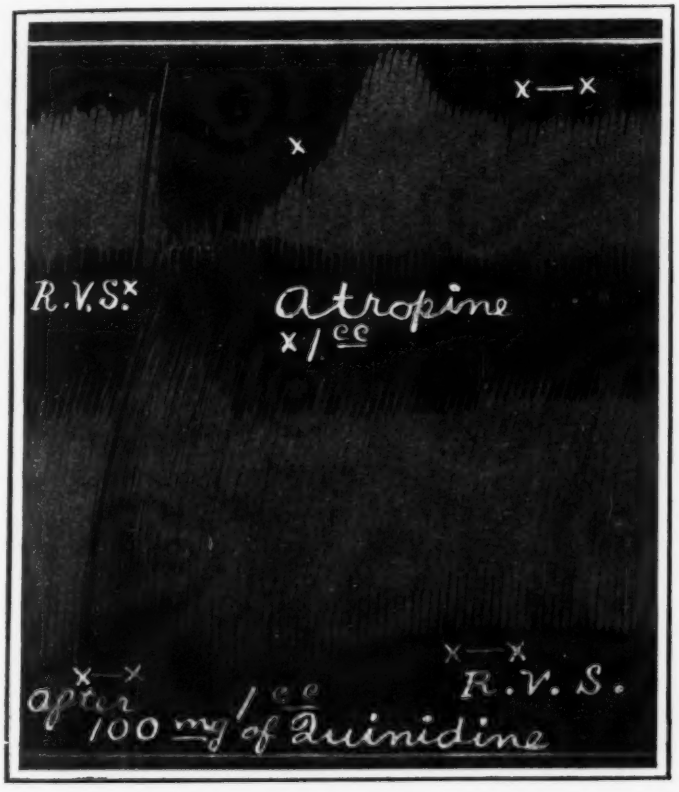

Fig. 16.

endings and then to determine by electrical stimulation of the nerve in the cervical region whether inhibition of the heart could still be produced. At the point indicated in the left of the tracing the right vagus nerve was stimulated and a prompt complete inhibition of the heart was produced. This lasted longer in the auricle than in the ventricle, which presently broke through the inhibition, but still showed its effects while the stimulation was continued. Following this, and while the inhibitory effect was still well marked in the auricle, an injection of 1 milligram of atropine was given by way of the femoral vein and this promptly stopped the lingering inhibitory effects in the auricle which responded by a marked increase in both rate and amplitude of its beat. Following this the right vagus nerve was again stim-

ulated, but no inhibitory effects were produced on the heart. We conclude from this (and other similar experiments) that not only are the vagus endings not paralyzed by quinidine, but that the inhibitory action of the vagi on the heart is, if anything, actually increased by quinidine. And we suspect that this action is of immediate importance in the matter of the checking of auricular fibrillation or flutter by quinidine.

Fig. 17.

This brings us to another phase of the action of quinidine. We have regularly observed that when an intravenous injection of adrenaline is given to a normal animal and a blood pressure tracing is made, then if one or two injections of quinidine be administered and this be again followed by adrenaline, it will be found that the animal is strikingly less sensitive than at first to the action of the adrenaline. Indeed this result is so marked that when we first observed it we were inclined to suspect that quinidine possessed a paralyzing action on the sympathetic nerve endings similar to that of ergotoxine. And we suspect that large doses of quinidine do actually depress the vascular sympathetic nerve endings. Such an action probably also occurs in the case of the sympathetic nerves to the heart. And this may be of considerable importance in lessening the irritability of fibrillating or fluttering auricles in clinical cases. This action on the sympathetics is best seen, however, in the blood pressure. For after large or repeated doses of quinidine, adrenaline may be

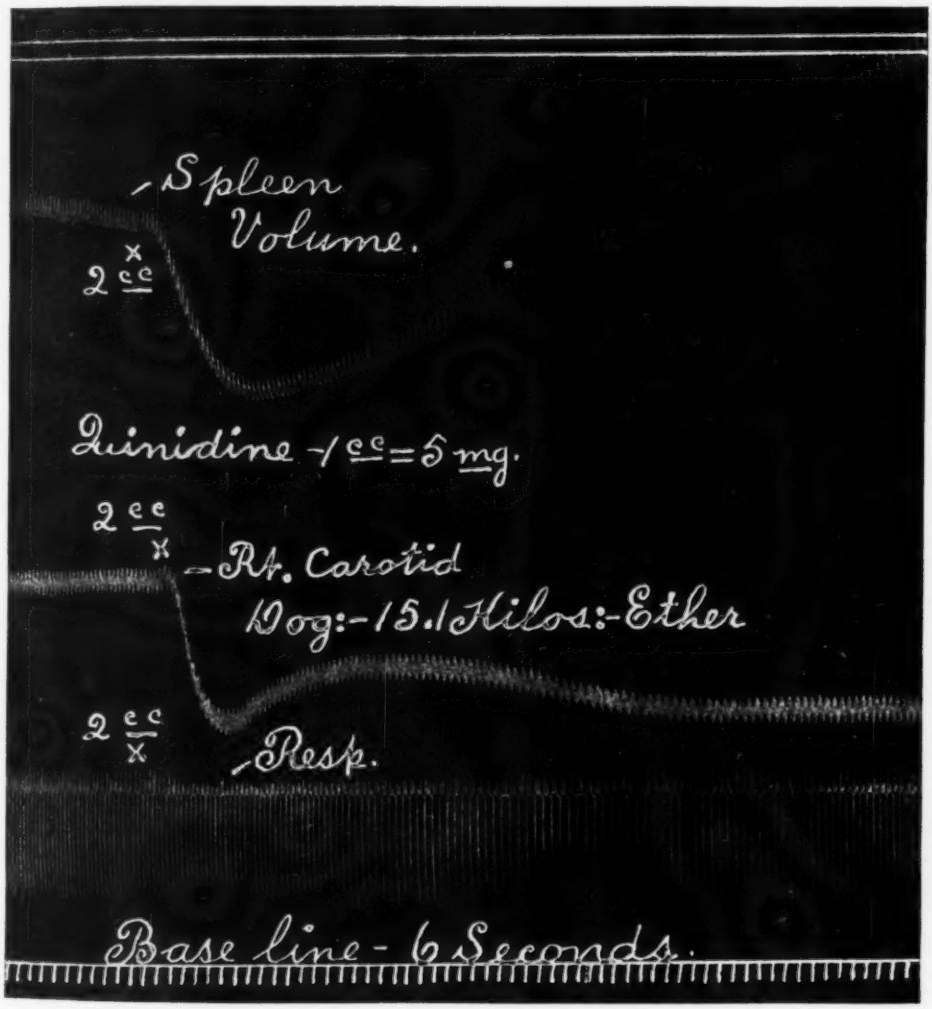

Fig. 18.

found to be very much less effective in producing a rise in pressure than it had been before the quinidine was administered. There are other features to this problem, however, and we may defer its further consideration until we discuss the peripheral action of quinidine.

Figs. 17 and 18 show the action of quinidine on the respiration, blood pressure and the volume of the kidney and spleen when the drug is injected intravenously. It will be noted that both the kidney and the spleen shrink in volume. We suspect that this is due to at least two causes. First, the general fall in blood pressure allows the kidney and spleen to shrink somewhat by their own elasticity. And further, the fall in systemic pressure sets

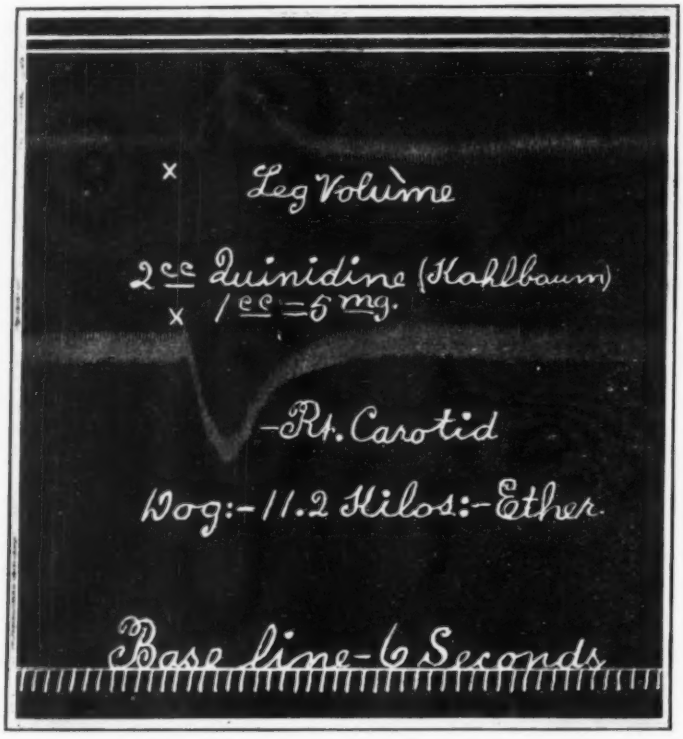

Fig. 19.

up a certain amount of asphyxia in the medullary vasomotor center. This center will respond by contraction of the visceral vessels and this action is probably concerned in the decrease in volume of the kidney and spleen as here shown. We believe (although we have not yet proved) that there is no *direct* action on the kidney or spleen which would tend to cause them to contract.

Figs. 19 and 20 illustrate another point in the general action of the drug. In these records the volume of the hind limb is recorded in the upper tracings. It will be seen that the leg volume increases while the general systemic blood pressure falls when quinidine is injected. This peripheral dilatation we believe probably plays a rather important part in the action of the drug.

We are not sure as yet whether this dilatation of the general systemic vessels as seen in the leg volume represents a central or a peripheral effect, but certain other features of the drug's action lead us to presume that quinidine here acts primarily on the peripheral vessels, and possibly to a considerable

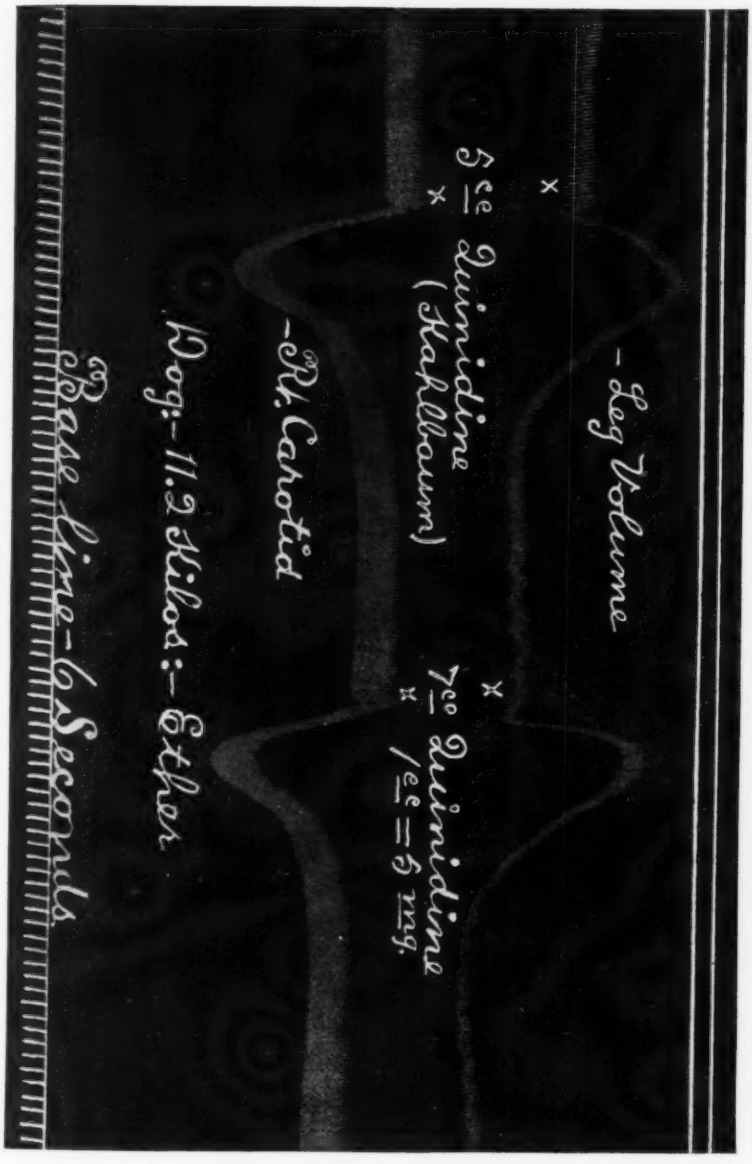

Fig. 20.

extent on the capillaries. We are at present carrying on further investigations along these lines. It was mentioned above that quinidine tends to decrease the sensitivity of an animal to adrenaline. If the drug should do this by slightly depressing the vascular sympathetic endings we would naturally expect to get a dilatation of the peripheral vessels when the drug is injected unless some counteracting influence, such as medullary asphyxia, should be

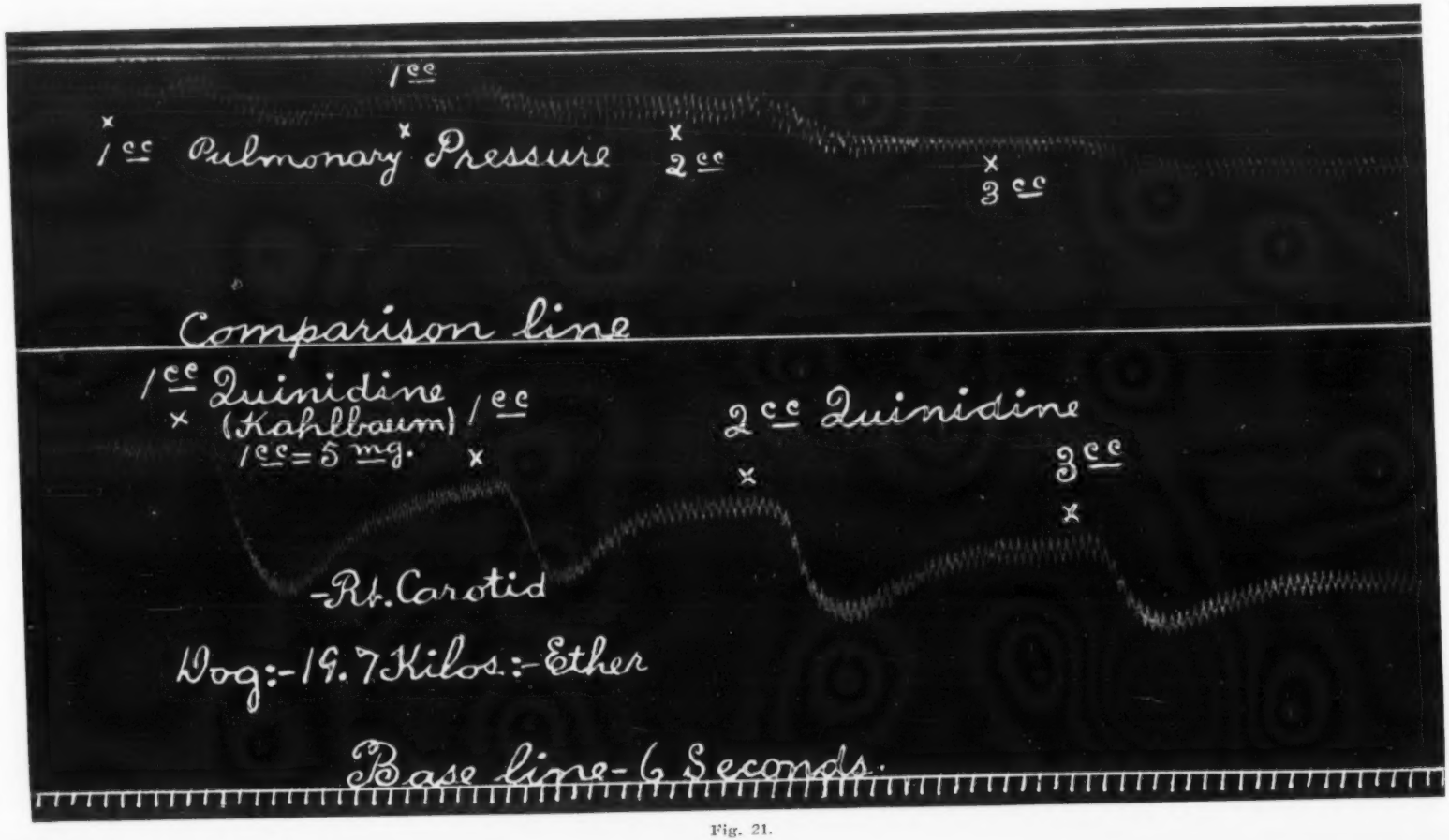
1 cc
1 cc Pulmonary Pressure 2 cc
3 cc
Comparison line
1 cc Quinidine (Kahlbaum) 1 cc
1 cc = 5 mg.
2 cc Quinidine
3 cc
-Rt. Carotid
Dog:-19.7 Kilos.:-Ether
Base line-6 Seconds.

Fig. 21.

brought into play at the same time. It is of interest to mention that a dilatation of the coronary vessels might be of considerable advantage to the heart in many clinical cases. Of course, the muscle fibers of the arteriole walls may be directly depressed by the drug, but it is our impression that the peripheral action of the substance is of greater extent and importance than this. We should like to suggest that a careful clinical study of the general condition of the *peripheral circulation* be made in all patients to whom the drug is given, both before and after the administration. We believe that such investigations

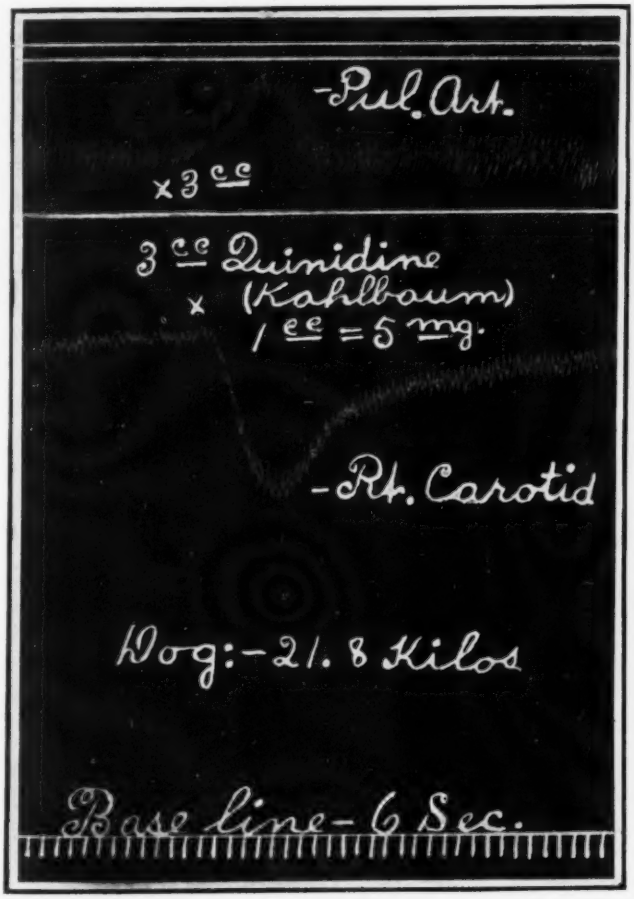

Fig. 22.

may throw considerable light on the question of the proper selection of cases for quinidine treatment.

The pulmonary blood pressure occupies a peculiar relation to the general circulation in cardiac disturbances, such as auricular fibrillation or flutter, and especially so when these conditions are further complicated by valvular disease. Consequently any drug which exercises a considerable action on the pulmonary pressure would be worthy of careful study when used in the treatment of such cardiac disturbances. As will be seen from Figs. 21, 22,

Fig. 21.

23, 24 and 25, quinidine exercises a marked effect on the lung circulation. Apparently a number of factors are involved in this action. Small doses rather tend primarily to produce a rise in the pulmonary pressure, while large doses always cause a fall, and in many cases this fall is out of proportion to that

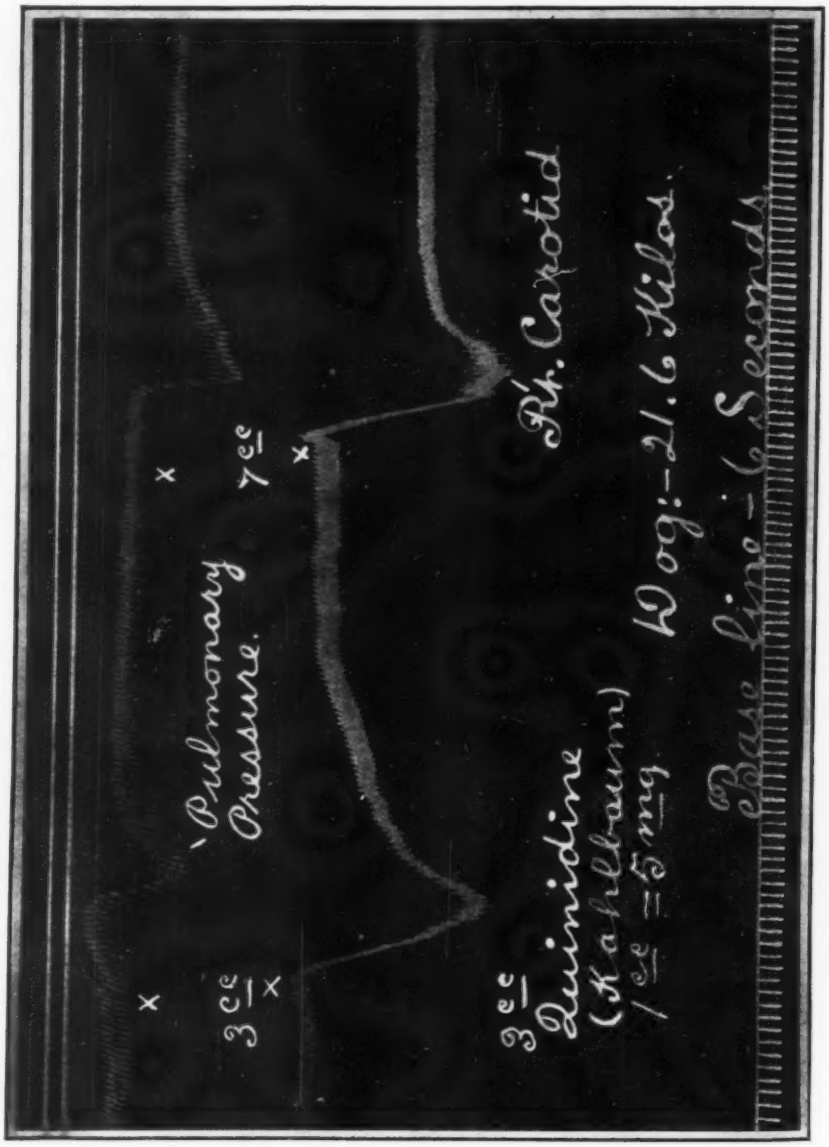

Fig. 23.

which is correspondingly produced in the systemic pressure. We believe that the slight rise produced in the pulmonary vessels by very small quantities is a secondary effect and occurs as a result of the fall in systemic pressure, or by a damming back of the blood in the heart. It is probable also that in some cases the early acceleration, and even stimulation, of the heart such as is shown in Figs. 1 and 2 may so affect the right ventricle that a slight

increase in the volume of blood thrown into the pulmonary arteries may occur for a brief interval. It seems certain, however, that larger doses of quinidine injected directly into the circulation will always produce a fall in pulmonary pressure. Obviously if any such action as this should occur clinically it might have a great deal to do with the outcome of the treatment. We have tried to reduce the conditions of our experiments as nearly as possible to those of the patient who may be treated with quinidine. Of course, the normal tissues and organs of a patient with a cardiac lesion will be affected by the adminis-

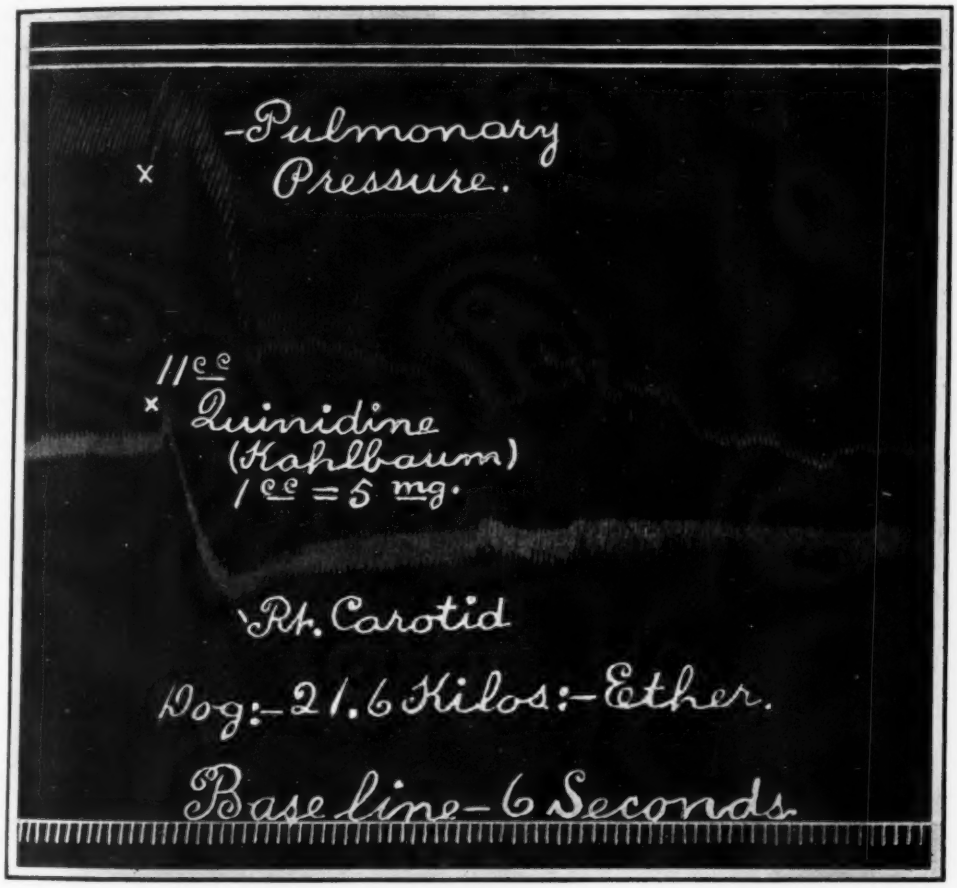

Fig. 24.

tration of quinidine in a manner analogous to that which would occur in a normal individual. And through a gradation of dosage we have here attempted to find out whether it is probable that a fall in pulmonary pressure occurs in the clinical use of the drug. We have recorded the pulmonary pressure by means of a water manometer connected with the left pulmonary artery. The distal limb of the manometer was connected by rubber tubing to a long *inverted* U-tube, filled with air and the distal end of the U-tube was connected with a recording tambour which marked on the drum. This arrangement gave a considerable magnification of the pulmonary pressure

over that of the systemic record which was obtained by means of a mercury manometer.

The proportionate dosage which we have used to obtain pulmonary pressure records has often been below that which is generally used in clinical practice, as indicated by comparing the weight of the animal with that of a patient. Absorption from the stomach or small intestine, of course, requires much more time than does intravenous injection to get the drug into the general circulation. And the regular fall in systemic pressure which the drug produces may have some indirect effect on the lowering of the pulmonary pressure. Judging from other results the depression of the heart muscle, which has generally been supposed to be the chief action of the drug on the circulation, is not sufficient to account for all of the fall in the pulmonary pressure. It seems evident, therefore, that a dilatation of the pulmonary arterioles and capillaries occurs under the action of quinidine. And we believe that this action in all probability occurs in many, if not in all, cases in which the drug is used clinically. Such an action would probably be of benefit in many instances, even if the drug had no effect on the heart at all. On the contrary, in other cases such effects might be of no benefit, or might even be harmful. But it is to be noted that the dilatation of the vessels of the hind limb with a consequent increase in volume, and the dilatation of pulmonary arterioles and capillaries both indicate that the drug possesses a distinct peripheral action on the circulation which should be taken into account in its clinical application.

In conclusion we must refer briefly to a point which has recently been especially emphasized by Lewis[7, 34, 42] and others with reference to the nature of auricular fibrillation and its treatment by quinidine. The circulation of contraction waves in muscle (Cassiopea) was studied by Mayer[36] as early as 1908. W. T. Porter,[33] in 1894, originated the block hypothesis of fibrillation. But later Garrey[31, 30] suggested that fibrillation of the heart muscle probably consisted essentially in a large number of small contraction waves circulating diffusely and simultaneously in many directions and entirely without coordination in the heart muscle. These waves were supposed to be conducted directly from muscle fiber to muscle fiber, and the refractory state of fibers which had just relaxed set up blocks against the waves in many places and thus diverted them promiscuously into other channels from which the refractory state had already disappeared. Mines[35] in particular studied this phase of the problem. These views regarding the essential nature of fibrillation have been generally held for a long while. But recently Lewis[7, 42] and his coworkers have introduced the view that in auricular fibrillation a single contraction wave only exists, and that this "is propagated and revolves perpetually upon a re-entrant path. The circus movement is repeated on the average 450 times a minute, and it is this circulating wave which, in its revolution, alone controls the beating of the auricle." The wave as thus conceived would be influenced by three factors "(a) the length of the path followed, (b) the duration of the refractory period at any given point; and (c) the speed at which the wave is conducted." Lewis[7] has suggested that

quinidine may act on auricular fibrillation by either slowing the rate of conduction of the contraction wave (thus tending to increase the length of the gap between the advancing crest of the wave and the retreating area of refractory tissue over which the wave has just passed and into which the

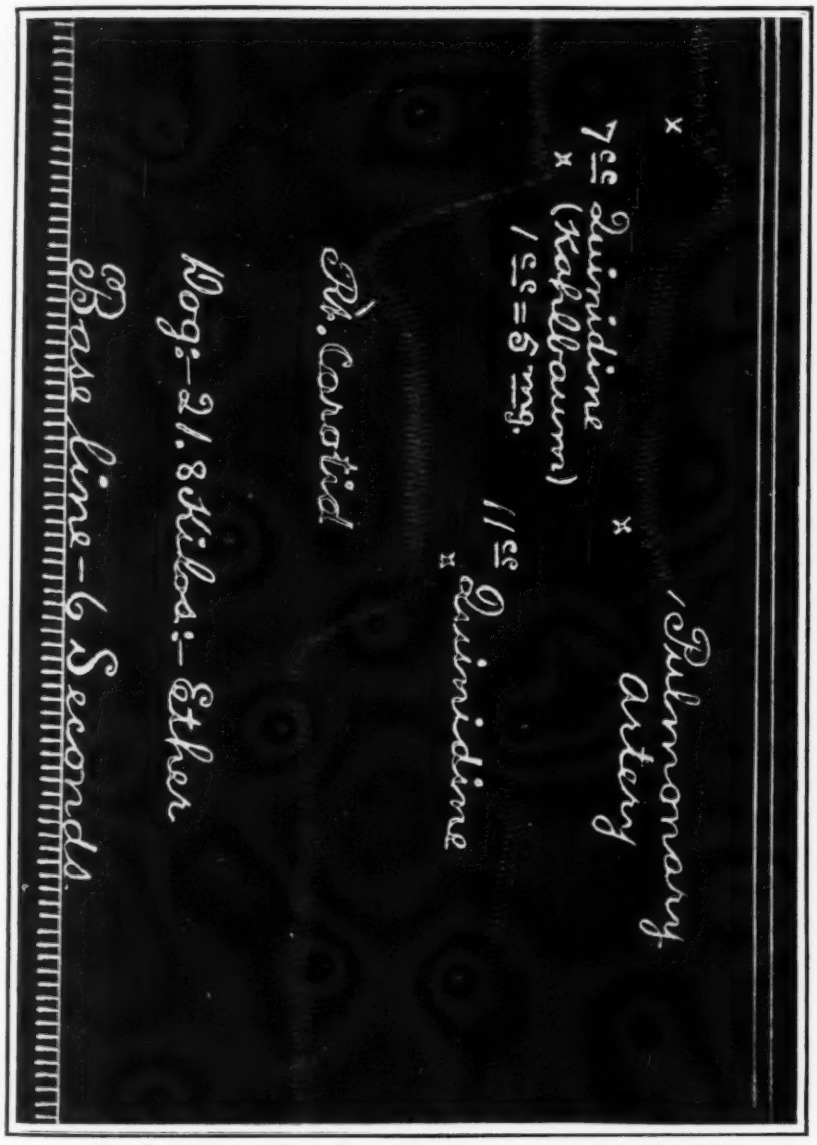

Fig. 25.

contraction wave is progressively reentering), or else by lengthening out the extent of the refractory period in tissue over which the wave has just passed. The first of these actions would tend to prolong the gap between the refractory tissue and the advancing crest of the returning wave and thus favor continuation of the fibrillation. The second action, however, by prolonging the refractory period in tissue which had just relaxed would tend to stop the

fibrillation because the gap between the refractory tissue and the oncoming crest of the wave would be shortened, and if this shortening should become great enough, the crest would meet refractory tissue and the circulating wave would thus be stopped. Presumably the auricle would then be in position to take up its normal beat. Lewis suggests that these two opposing actions may neutralize each other so far as beneficial action on the heart is concerned, or the slowing of the conduction may even make the condition of the beat worse. He also states that (unpublished) experiments have shown that quinidine in doses comparable to those used clinically may lengthen the refractory period by as much as 50 per cent or more.

We are of the opinion that from a pharmacological standpoint this attractive hypothesis is inadequate to explain the whole of the action of quinidine on the heart. And we should not be at all surprised if later work should render it entirely untenable. When we first approached this problem we naturally suspected that there was nothing unique in the action of quinidine on auricular fibrillation, but that perhaps a large number of drugs which exercise a general depressant action on the cardiac muscle would in all probability act in a very similar manner. It appeared to be simply a question of selecting a substance of sufficiently low general toxicity, and one which would be eliminated from the blood but slowly in order that a prolonged, mild depression of the auricular tissue might be produced. The drug undoubtedly acts on both ventricles and auricles. It tends to counteract the development of fibrillation of the ventricle after such drugs as aconitine or digitoxin. And one of the earliest actions on the normal heart consists of a slight dilatation of the ventricle as shown by the cardiometer. The checking of fibrillation of the ventricles[32] in perfused hearts by the temporary addition of small quantities of potassium chloride solution to the perfusion fluid indicates also that quinidine does not possess a unique action in this regard. And it is our surmise that in due course it will be found that quinidine acts on the musculature of the peripheral vessels, and perhaps even on the skeletal muscles, in a manner quite similar to that in which it acts on the heart muscle. Such an action on the coronary vessels would probably be of importance in some, and perhaps in many cases. And the decrease in the power of adrenaline, when injected intravenously, to raise the general blood pressure after quinidine may indicate an action on the sympathetic innervation, although we cannot at present rule out the possibility that this may be due to an action on the capillaries. Slight changes in the pulmonary blood pressure by an action on the pulmonary arterioles or capillaries might also be of importance in many cardiac cases. And finally, as a word of caution, it is interesting to note that Withering introduced digitalis into the Edinburgh Pharmacopoeia in 1783, and in 1785 he published an article on the *Abuse* of the Fox-glove. And the year 1922 finds us still willing to learn more than we at present know about the fox-glove. Hence a word to the wise will be sufficient with reference to the too hasty formation of conclusions regarding the action of quinidine.

The following bibliography, while not entirely complete to date, will be sufficient to enable those who are interested to follow the question still further.

BIBLIOGRAPHY

[1]Wenckebach, K. F.: Ueber Chinin als Herzmittel, Berl. klin. Wchnschr., 1918, lv, 521.
[2]Wenckebach, K. F.: Die unregelmässige Herztätigkeit und ihre klinische Bedeutung, Leipzig, 1914, Engelman.
[3]Frey, W.: Berl. klin. Wchnschr., 1918, lv, 417, 451.
[4]Levy, R. L.: Jour. Am. Med. Assn., 1921, lxxvi, 1289, May 7.
[5]Santesson, C. G.: Arch. f. exper. Path. u. Pharmakol., 1893, xxxii, 327.
[6]Hofmann, F. B.: Ztschr. f. Biol., 1915, lxvi.
[7]Lewis, T., Drury, A. N., Iliescu, C. C., and Wedd, A. M.: Brit. Med. Jour., Oct. 1, 1921, ii, 514.
[8]Mackenzie, J.: Brit. Med. Jour., Oct. 8, 1921, ii, p. 576.
[9]Orr, J.: Brit. Med. Jour., Oct. 8, 1921, ii, p. 576.
[10]Von Bergmann: München. med. Wchnschr., 1919.
[11]Frey, W.: Deutsch. Arch. f. klin. Med., 1921, cxxxvi, 70.
[12]Faber, K.: Ungesk. f. Laeger., May 5, 1921, lxxxiii, 577.
[13]Hecht, A: Wien. klin. Wchnschr., 1917, vi, 171.
[14]Boden, E., and Neukirch, P.: Deutsch. Arch. f. klin. Med., June, 1921, cxxxvi, p. 181.
[15]Wiechmann, E.: Excretion of Quinidine in the Urine, Ztschr. f. d. ges. exper. Med., 1918, vii, 155.
[16]Hewlett, A. W., and Sweeney, J. P.: Jour. Am. Med. Assn., Dec. 3, 1921, lxxvii, 1793.
[17]Hamburger, W. W.: Jour. Am. Med. Assn., Dec. 3, 1921, lxxvii, 1797.
[18]Oppenheimer, B. S., and Mann, Hubert: Jour. Am. Med. Assn., Dec. 3, 1921, lxxvii, 1800.
[19]Hecht and Rothberger: Ztschr. f. d. ges. exper. Med., 1918, vii, 135.
[20]Schott, E.: Deutsch. Arch. f. klin. Med., February, 1920, cxxxiv, 208.
[21]Klewitz, F.: Deutsch. med. Wchnschr., Jan. 1, 1920, xlvi, 8.
[22]Benjamin and von Kapf: Deutsch. med. Wchnschr., 1921, xlvii, 10.
[23]Jenny, E.: Schweiz. med. Wchnschr., March, 1921, li, 272.
[24]Drury, A. N., and Iliescu, C. C.: Brit. Med. Jour., Oct. 1, 1921, ii, 511.
[25]Wolferth, C. C.: Am. Jour. Med. Sc., December, 1921, clxii, No. 6, p. 812.
[26]Eyster, J. A. E., and Fahr, G. E.: Arch. Int. Med., Jan. 16, 1922, xxix, No. 1, p. 59.
[27]Leschke and Ohm: München. med. Wchnschr., 1921, lxviii, 65.
[28]Wisser: Inaug. Dis., Cologne, 1920.
[29]Clerc, A., and Pezzi: Paris Méd., Dec. 3, 1921, xi, 440.
[30]Garrey, W. E.: Am. Jour. Physiol., 1914, xxxiii, No. 3, 397.
[31]Garrey, W. E.: Interstate Med. Jour., 1921, xix, 1081. Proceedings of the St. Louis Medical Science Club for the meeting held Nov. 12, 1912.
[32]Erlanger, Joseph: Am. Jour. Physiol., 1912, xxx, 400.
[33]Porter, W. T.: Jour. Physiol., 1894, xv, 135; Am. Jour. Physiol., 1899, ii, 129; ibid., 1905, xiii, pp. XXIII and XXIV; Ibid., 1905, xv, 5.
[34]Lewis, T.: Heart, 1910, i, 353.
[35]Mines, R. G.: Jour. Physiol., 1913, xlvi, 349.
[36]Mayer, A. G.: Popular Science Monthly, December, 1908, p. 481.
[37]Romanes, G. J.: Jelly Fish, Star Fish and Sea Urchins, 1885, p. 67.
[38]Sappington, S. W.: Jour. Am. Med. Assn., Jan. 7, 1922, lxxviii, 59.
[39]Editorial: Jour. Am. Med. Assn., 1921, lxxvii, 1822.
[40]Floystrup, G.: Ungeskr. f. Laeger, Oct. 27, 1921, lxxxiii, No. 43, p. 1389.
[41]Abrahamson, L.: Dublin Jour. Med. Sc., 1921, iv, 562.
[42]Lewis, T., *et al.*: Heart, a series of articles published in 1920 and 1921.

BERIBERI: SOME FACTS AND THE POSSIBLE HYPOTHESIS

By Tsang G. Ni, M.S., China

THERE are different names for beriberi and many theories have been offered concerning the etiology of it. "Che-Zee" is the term given to a disease resembling the "wet" form of beriberi in an old Chinese medical book, "Soo-Van" (said to have been written about 2500 B.C.). Since the Don-Dynasty, the term "Char-Chee" has been seen in literature. The Chinese word "char" means foot. The second word "chee" means gas or something which is labile. It has been described in old literatures that there are two varieties of "chee": "inm" (negative or cathodic) and "yarnn" (positive or anodic); and that in the healthy body there is a properly antagonistic, cooperative and harmonized condition among "inm," "yarnn" and circulation. Beriberi was regarded to be due to the unbalanced antagonism of "chee" in the circulation, starting its symptoms in the "char" (foot). In modern times being convinced by the work done in different countries and the cases in the Chekiang Army Hospital, Chinese medical students and instructors have believed that the deficiency in antineuritic vitamine is one factor causing beriberi.[1]

As stated in literatures of different countries, beriberi has been attributed to the lack of harmony between "chee" and circulation to weakened kidneys;[2] to moist weather; to unfit water and soil; to night air;[3] to poisoning by arsenic, by carbon dioxide, by oxalate, by rice, by fish; to a helminth infection; to autointoxication; to nitrogen starvation; to some protozoon infection with a possible insect-carrier; to the phosphorus starvation; to a bacterial infection in the body; to fungi; to a bacterial toxin of an extracorporeal origin. It is not necessary to deal at length with many ancient theories which can be disproved easily.

Among those who have favored the theory that the disease is an infectious one and not due to deficient nutrition may be mentioned: Scheube,[4] Manson,[5] Balz,[6] le Dantec,[7] Jeansalme,[7] Marchoux,[7] Daniels, Wright, Castellani, and Schibayama.[8] Schibayama states that it is not unreasonable to assume that the microorganisms of beriberi are only present in the Orient and, given a predisposing cause, are capable of producing disease. Castellani[9] also holds a somewhat similar view.

To summarize from the evidence, it appears more likely that a parasite will be found to be a spreader of the disease, which makes it more probable that the actual cause will be found to be a protozoon than that it is due to diet.

Marchoux, writing in 1910, believes that a diet of white rice furnishes in the intestine a favorable culture medium for the development of the organism of beriberi, and that the addition of rice bran to the diet renders the condi-

tion in the intestine unfavorable for the development of this organism. There are numerous other investigators who have described various species of protozoa or of bacteria as the cause of beriberi. However, these have not been definitely proved by scientific researches.

In the fall of 1916 there were about two hundred cases of beriberi in the Chekiang Army Hospital. The bacteriologists in the hospital failed to isolate either specific species of bacteria or of protozoa except those well known as the causative agents of other diseases. However, the fact that the causative microorganism for the disease has not been discovered is not a final argument against its infectious nature, for the specific organism in many diseases of an undoubted infectious nature has been sought for as diligently and as unsuccessfully as in the case of beriberi. We have known that there are microorganisms that probably cannot be seen, at least not today, with even the highest powered microscope, and that there are viruses able to pass through the pores of the filters. Shiga and Kusama[10] attempted to obtain the reaction of deflection of the complement in the serum of beriberi patients. The result of their experiments was negative. Grijns and de Haan[11] made experiments to demonstrate the presence of antibodies in the blood of organs of beriberi cases or of fowls suffering with polyneuritis; they were no more successful than we. Riddell, Smith, and Igarevidez stated that cultures made from the heart and lungs at postmortem were negative. No parasites were found in the ileum.[12]

Although we do not want to exclude definitely the possibility of the disease being an infectious one, merely by means of negative experiments, yet, if by scientifically controlled experiments we can produce the disease and exclude the influence of a living specific microorganism, the infectious theory of its origin can be regarded as no longer tenable. Eykman found that fowls develop polyneuritis when fed on polished rice. Grijns,[14] Sakaki,[15] and others repeated and verified the experiments.

Concerning the relation of polyneuritis gallinarum to beriberi there are different opinions. Kusama and Shiga[10] believe that it would be too much to say "The polyneuritis of animals and beriberi are identical." Shaumann[16] states "Not that both diseases are identical but there seem to exist reasons for assuming that both are related to each other." On the other hand Vedder and Chamberlain[17] said that both are essentially identical.

Besides the argument of identity of these two diseases, the metabolism of the human body is different from that of birds. In birds the urea is replaced by uric acid. One may say that without similar experiments or observations on man the results obtained with fowls would not be applicable to man.

Let us consider the observations upon the production of beriberi in the human body. Fletcher[18] states: At the commencement of the experiment all patients showing symptoms of beriberi were removed to the district hospital. The result was that 34 out of 120 persons fed on uncured (Siamese) rice suffered from beriberi and 18 died, while among 123 patients dieted on cured rice there were no deaths from beriberi. Kiewiet de Jouge[11] made

experiments on 384 patients. Katjang idjo was given to 182 of them, but not to the remaining 202. The result was as follows:

	With katj. idjo (per cent)	Without katj. idjo (per cent)
Suffered from beriberi and remained unchanged	15.0	23.4
Improved	75.0	13.3
Became worse	10.0	63.3
Died	2.5	30.0

Takaki succeeded in eradicating beriberi from Japanese Navy by allowing a large portion of nitrogenous food; he believes that beriberi is due to nitrogen starvation, that is, the disproportion of nitrogenous and nonnitrogenous elements. As to the nitrogen equilibrium many experiments were made in different laboratories. The average of 109 experiments belonging to twenty-five investigations[19] showed an indicated requirement of 0.635 gm. of protein per kilo of body weight. It was estimated that the diet given to the people in Bilibid prison consisted of: 97.17 gm. of proteins, 17.24 gm. of fats, 491.04 gm. of carbohydrates and 26.52 gm. of salts. Yet the outbreak[9] of beriberi in that prison was not prevented. On the other hand, there were no deaths from beriberi in Culion, where the deaths from beriberi in previous years were 309, during the interval following the use of unpolished rice.[20]

Not only the theory of nitrogen starvation may be disproved by these observations, but also the deficiency in some particular,[21] growth-promoting amino-acids may be excluded. The chief protein of rice is oryzenin,[22] a glutelin. Fuji and others[23] have determined some of the amino acid cleavage products of this protein. The analyses indicate that the protein is regarded as "complete." Moreover human beriberi may also be caused by diets which do not include polished rice, as shown from the observation of Axel Holst,[24] of Little,[25] and of Lavolaoe.[26]

With reference to the nature of the influence of rice causing beriberi some theories other than vitamine deficiency may be mentioned. Braddon[29] concluded: That beriberi was not an infectious disease but a form of food poisoning. He suggested that the cause was not indeed rice, but diseased rice, rice with a poison derived from decay due to some fungus, or mould, or spore. Considering Braddon's idea Fraser and Stanton carried out experiments in an isolated district. The blood and urine of patients suffering from beriberi were examined, but in no instances were special organisms found. The results lend support to the view that beriberi has at least a relationship with the consumption of a certain particular sort of rice. Eykman concluded[13] that a poison existed in rice and for this poison something in the pericarp was an antidote. After arriving in Holland he made some more experiments and again concluded[27] that the poison was present in starch or developed from it. Eykman's poison theory was disproved by Grijns, who succeeded in causing polyneuritis in fowls by feeding them on food without starch, and concluded that the nerve system requires unknown constituents of food. He injected into the peritoneal cavity of fowls the blood of diseased

birds (a maximum of 220 cubic centimeters in 23 injections), but with negative results.

"Phosphorus starvation" is another theory in regard to the etiology of beriberi. Aron[28] concludes that foodstuffs which are poor in phosphorus (phytin) have been shown to cause beriberi. With reference to phosphorus requirement of man, the survey made by Sherman[30] deserves notice. The data from ninety-five experiments range from a minimum of 0.52 gm. to a maximum of 1.20 gm. with an average of 0.88 gm. phosphorus per 70 kg. daily. The minimum requirement of phosphorus is rarely approached. Moreover Simpson and Edie[31] found that the disease was neither cured nor prevented by the organic phosphorus compounds. The relation may be such, that rice with a phosphorus pentoxide (P_2O_5) content below 0.4 per cent is deficient in vitamines too, as concluded by Stanton and Fraser.[32]

It is not merely a theory but a fact that deficiency in certain substance, "vitamin" (or vitamine) is, if not the only one, at least one factor in causing beriberi. For there are so many feeding experiments, clinical observations, and chemical investigations which lend the same support to the vitamine causation, that it seems to be unnecessary to quote them in detail. Funk[33] has discovered a method to isolate the alcohol-soluble protective substance from yeast, rice-polishings and other stuffs. He considered this substance a pyrimidine derivative. In addition to Funk's base Vedder[34] has reported the isolation of two groups of substances (purine bases, choline-like bases) from rice-polishing, which are capable of partly protecting fowls. Both Funk's base and Vedder's bases are found in undermilled rice, but the starchy part of cereal food is poor in the "antineuritic vitamine." This explains the clinical cases as well as the feeding experiments. In Fraser and Stanton's study, beriberi appeared among the members of the party receiving polished rice but not among the party eating undermilled rice; after reversing the food of both parties the conditions were reversed.

Clinical cases give us no less convincing explanation than the laboratory experiments. Heiser[35] reports 50 cases of beriberi cured (except two very advanced cases) by giving daily 15 grams of rice polishings. Danier[36] at Sargon reports the efficiency of the preventive use of rice-bran. William and Saleeby[37] state that in 5 of 6 cases in which vitamine was prescribed, improvement was very radical. These are in general accord with the results on Chinese soldiers, as I have noted in the Chekiang Army Hospital.

As to the treatment and prevention of beriberi, there are nearly as many theories as concerning its etiology. Our very ancient people, believing the theory of "Chee-Nih" (improper antagonism of gas in circulation), developed a custom as a means of prophylaxis. They thought that "nien-wei" or "nooh" (a kind of fancy dressing parade), arousing, stimulating and amusing the mass of people, might relieve and neutralize the ill effect of inactive and depressive life, which was supposed to cause "Chee-Nih." However, some of them knew that "nooh" would be carried out more efficiently if the common people believed it to be God's will. That is why "nooh" was considered religiously and its purpose of preventing beriberi and other diseases,

as proposed by a few of the ancient initiators and described in an old book, "Chu-Ree," was not understood.

Since various methods to extract vitamines from rice-polishings and other stuffs have been developed, the etiology, the treatment and the prevention have gained a further step. The administration of Funk's base (Funk's vitamine) is followed by prompt improvement,[34] which was not known before Funk's work. The administration of unhydrolized extracts obtained from 1 kilogram of rice-polishings results in the immediate dissipation of edema. The case of infantile beriberi (of the "wet" type) has been cured[34] by giving that portion of extract which does not contain Funk's base but contains bases of other kinds. If the autolyzed yeast extract is used, adults are given from 15 to 40 c.c. three times a day; children may be given from 2 to 4 c.c. every three hours.[38]

DISCUSSION

Comparing what has been done by others and what I have seen in Chekiang the following hypothetic possibilities may be considered. The minimum vitamine, i.e., such amount of vitamine that is necessary to maintain normal metabolism without showing beriberi symptoms, probably differs in individuals. In the same individual it may vary in different tissues, and may be different from time to time when condition and metabolism are changed. The beriberi symptoms would appear when one or several of the following conditions should occur:

Increase in the elimination or destruction of the specific vitamine (or vitamines).

Decrease in the intake of vitamines.

Decrease in the intake of vitamine-building materials (if the body cell can manufacture vitamines).

Attachment of vitamine to certain tissue and thus retained, or rendered inactive by combining with other substances.

Decrease in the capability of body cells to produce vitamines.

Decrease in the capability of body cells to utilize vitamines.

Increase in the demand of vitamine so that the normal minimum amount is not sufficient to meet the need.

Under certain conditions the beriberi symptom does not appear even if the vitamine content in the body is below the normal threshold. For instance, the administration[39] of calcium lactate prolongs the period required for the development of neuritis. This is probably due to the fact that in the presence of calcium lactate the vitamine may be used up to a degree below the normal minimum. Clark believes that this has permitted degeneration to occur in certain fibers of the sciatic nerve to a degree much more intense than has been observed for fowls fed on rice alone.

We do not know the nature of vitamine action in normal metabolism or in beriberi. Neither do we know to what extent vitamines are catabolized and eliminated daily. Some believe that they play a rôle which might be likened to that of the hormones in the cellular activity or the opsonins and other antibodies in the infection. Veogthin and Myers[40] say that extracts

obtained from brewers' yeast cause an increase in pancreatic secretion when injected intravenously. Secretin solutions obtained from the duodenum of the hogs contain a considerable amount of antineuritis vitamine, as shown by their power in relieving the paralytic symptom of polyneuritic pigeons. Starling points out that harmones should either be easily destroyed or readily excreted. Their destruction is influenced by different conditions. Although the identity of the water-soluble vitamine B and secretin has been denied,[50] it is not impossible that the destruction of vitamine also is influenced by some factors.

Either decrease in the vitamine-building material or in the capability of body cells affects the manufacturing of vitamines in the body. It is not known whether the human body-cell can synthesize Funk's base (vitamine) from nonvitamine materials. It is known that secretin and other harmones are formed in the body.[41] Some claim that[40] the chemical and physical properties of secretin and vitamine are similar. They might be derived from histidin by splitting off carbon dioxide, or the one might be derived from the other by rearranging the atoms in the molecule. Funk,[33] Williams and Saleeby[42] report that allantoin (0.1-0.4 gm. three times daily) has a beneficial effect on certain cases of beriberi. The latter suggests that allantoin resembles Funk's vitamine in its chemical constitution. From these statements it appears to be that either the body cells manufacture vitamines as they do in the cases of secretin and allantoin, or they use them as raw materials. It also appears to be not improbable that normally beriberi does not occur, because the vitamine equilibrium is maintained by two sources: exogenous and endogenous. If one of the two, under certain conditions, is diminished and the other is not sufficient to compensate it, then the vitamine in the body is below its normal minimum (safe minimum) and beriberi occurs.

In the fall of 1916, there were about two hundred beriberi patients in the army hospital, when no beriberi cases appeared in the school hospital in the same city. The sanitary condition of soldiers was better than that of the civil laborers. Soldiers did feed on undermilled rice, vegetables and meat. Yet the outbreak was not prevented. As far as food was concerned, the vitamine content would have sufficed them if they had been in civilian life. As they had to take drill, their metabolic demands might be changed. Thus, standing in a rigid military position is said to increase creatinine excretion.[43] Vedder and Clark[44] state that chromatolysis and changes in the tigroid substance of the cells of the cord, similar to that of the pigeons which have been exhausted by long flights, may also be demonstrated in birds which have developed polyneuritis. This shows that muscular work affects the nerve system somewhat as the beriberi does, probably by exhausting vitamines. Soldiers, doing muscular work, possibly demand more vitamines; consequently the normal vitamine content of civilian life is not sufficient to meet the increased need of the soldier and thus to prevent beriberi. From the experience of the outbreak of beriberi in Kut, Hehir[45] concludes that the onset of beriberi was precipitated or accentuated by marching or any kind of severe continuous exercise. This may also be proved by the facts in Chekiang First

Division. A number of cases were checked either by allowing a rest before the soldier became exhausted or by giving him more vitamines than necessary for civilian life. We found that, although we did use undermilled rice, the vitamine content was insufficient to meet the increased demands. After giving a certain amount of unpolished rice and wheat and rice-bran, good results were shown.

It seems probably that the mental factor might play a no less important rôle in vitamine variation than the drill. The psychologic excitement might influence the vitamine metabolism by modifying the ability of body cells. By increasing a certain kind of internal secretion or by other means vitamine in the body might be destroyed more rapidly and abundantly; or, through a molecular rearrangement or a firmer union, its action might be changed and would not be utilized as usual. Or during mental excitement the nervous system or the body cells as a whole, being affected by internal secretions or other means, might lose the power of forming and utilizing the vitamines economically. In the case of emotional diabetes, for instance, mental excitement brings about an abundant output of adrenaline and an increased destruction of glycogen. McCarrison[49] has found that the suprarenals of beriberi patients undergo a marked hypertrophy. Sometimes the body cell may lose the ability of utilizing certain substances: in the absence of pancreatic internal secretion the body cells lose their capability of utilizing carbohydrates. Furthermore, it may also be shown by the following fact. Highet[46] reported that a Siamese police sergeant, who, having had a dispute with one of his subordinates, was put into a police cell to await trial. In thirty-six hours he became so ill that he was transferred to a hospital where he died in a few hours, with all the symptoms of acute, wet beriberi. In the winter of 1916 the term "char-chee" beriberi) was so familiar to the soldiers of the First Division that nearly a "beriberi-phobia" was in existence. There were three men who, doing some writing work, did not have drill, suffered from beriberi. As they were in a condition of fearing beriberi infection, their vitamine metabolism might be changed owing to the mental uneasiness.

Steenbock and Bontwell[47] have obtained evidence that under certain conditions the vitamine is very labile and under other conditions it may be stable. Williams[48] succeeded in preparing, synthetically, substances that would cure polyneuritis. These substances display a dynamic isomerism which has a profound influence on their antineuritic properties. The curative force of each compound readily undergoes at ordinary temperature a rearrangement of its atoms resulting in the formation of physiologically inert substance. By application of dry heat the noncurative form can be reconverted into a curative one. Although the synthetic substance in which this physiologically important isomerism was first discovered was hydroxypyridine, it now seems probable the antineuritic properties are not peculiar to them but are inherent in the type of isomerism of which they are capable. Such laboratory work has shown the probability that the utilization and activity of vitamine in the body might be diminished under certain conditions.

Deficiency in vitamine metabolism is not always accompanying the defi-

ciency in general nutrition. The body weight of our beriberi soldiers, at the beginning of the disease, was not less than when they enlisted. Their vital resistance was not always lowered, at least not at the beginning. This was shown by the fact that the majority of our beriberi patients did not have erosion about the corners of the mouth. While in the school hospital (where clinical training is given to medical students) our civilian patients, whose general nutrition condition was poor, as manifested by the sore mouth and tongue, and conjunctivitis, did not show beriberic symptoms.

SUMMARY

Disturbance of vitamine metabolism is one, if not the only one, factor causing beriberi.

The disease can be produced in such a way that the infectious theory of its origin can be regarded as no longer tenable.

The theory of nitrogen-starvation or of phosphorus-starvation may be discredited by comparing with the actual requirement.

The source of vitamine in the body might be endogenous as well as exogenous.

Muscular or mental strain might increase the vitamine requirement and assists to develop beriberi.

Under different conditions vitamine may be labile or stable, active or inactive, so that the same amount of vitamine in the body may not have the same antiberiberic function.

Under different conditions the capability of cells and the utilization of vitamine in the body might be changed, which would have influence on causing beriberi.

Many other theories of beriberi etiology, besides vitamine deficiency, can be disproved by experiments.

Deficiency in vitamine and deficiency in general nutrition may not always go side by side: beriberi may appear before the general condition breaks down and may not appear even after the disturbance of general nutrition.

REFERENCES

[1]Chien: Leeture on Disorders of Metabolism in Chekiang Province Medical School; also, Miscellaneous of Chinese Med. Students' Society, Vol. 1.
[2]A Book on Beriberi (by Toong of Soon-Dynasty).
[3]Malcolmson, J. G.: A Practical Essay on the History and Treatment of Beriberi, p. 38.
[4]Scheube: Die Krankheiten der Warmen Länder, 1908, p. 265.
[5]Manson: Tropical Disease, London, 1907, p. 367.
[6]Balz: Handbuch der Tropen Krankheiten, p. 146.
[7]le Dantec: Bull. Sec. path. exotique, 1910.
[8]Schibayama: Philippine Jour. Sc., Sec. B., 1910, p. 122.
[9]Castellani: Manual of Trop. Med., London, 1910.
[10]Shiga and Kusama: Beihefs z. Arch. f. Schiffe. u. Trop. Hyg., 1911, p. 15.
[11]Grijns and de Haan: Genees Tyda. v. Ned. Ind., 1909, p. 49.
[12]Riddell, Smith, and Igarevidez: Jour. Am. Med. Assn., 1919, p. 569.
[13]Eykman, C.: Anat, en Bakt., Batavia, 1896.
[14]Grijns: Med. Lab. Anat., 1900.
[15]Sakaki: Jour. Sei-i-Kwai, Mar. 31, 1903.
[16]Schaumann: Tran. Sec. Trop. Med. and Hyg., 1911, p. 589.
[17]Vedder and Chamberlain: Philippine Jour. Sc., Sec. B., 1911.
[18]Takaki: Lancet, 1916, l, pp. 1371, 1451.

[19]Hirschfeld: Arch. ges. Physiol., 1887.
—Hirschfeld: Virchows Arch. Path. Anat., 1888.
—Kempler: Ztschr. f. klin. Med., 1889.
—Viot: Ztschr. Biol., 1889.
—Pechsel: Eiweiesbedarf des gesunden Mensch., Dissertation, Berlin, 1890.
—Lapioquo: Arch. Physiol., 1894.
—Siven: Skand. Arch. Physiol., 1900, 1901. Ztschr. f. klin. Med., 1901.
—Jaffi: U. S. Dept. Agric. Bull., 1902.
—Glaessner: Ztschr. dist. physik Therap., 1903.
—Chittenden: Physiol. Economy in Nutrition, 1904.
—Aron: Biochem. Ztschr., 1911.
—Hindhede: Skand. Arch. Physiol., 1912, 1913, 1914.
—Fodor: Arch. ges. Physiol., 1914-15.
—Cooper: Biochem., 1917, Pope: ibid., 1918, xxxiv.
—Winters: ibid., 1918, xxxv. Sherman, Winters: ibid., 1919, xxxix. Sherman: ibid., 1920, xli.
[20]Jour. Am. Med. Assn., 1911, p. 1237.
[21]Hopkins: Sc. Prog., I, 1906, p. 159.
[22]Kajiura: Biochem. Jour., 1912, vi, 171.
[23]Jour. Coll. Agr., Imp. Univ., Tokyo, 1909, i, 77.
[24]Jour. Hyg., 1907, p. 619.
[25]Jour. Am. Med. Assn., 1912, lviii, 2029.
[26]Jour. Am. Med. Assn., 1912, lix, 2134.
[27]Jour. de Haan: Read at the first meeting of the Far East Assn. of Trop. Med., March 11, 1910.
[28]Aron: Philippine Jour. Sc., Sec. B., 1910, p. 97.
[29]Braddon: The Discussion of the Cause of Beriberi, London, 1911.
[30]Sherman: Jour. Biochem., Feb., 1920, p. 173.
[31]Simpson and Edie: Brit. Med. Jour., 1911, i, 1422.
[32]Stanton and Fraser: Lancet, 1915, i, 1024.
[33]Funk: Jour. Physio., 1912, xlv, 75.
[34]Vedder: Philippine Jour. Sc., Sec. B., 1913.
[35]Heiser: Jour. Am. Med. Assn., 1911, p. 1237.
[36]Ann. de l'Inst. Pasteur, Feb., 1911. No. 2.
[37]Philippine Jour. Sc., 1915, p. 117.
[38]Salesby, N. M.: Philippine Jour. Sc., 1919, p. 12.
[39]Gibson: Philippine Jour. Sc., 1913, p. 364.
[40]Jour. Am. Med. Assn., Sept. 13, 1919, p. 863.
[41]Fitch and Forty Contributors: Dietotherapy, 1918, p. 65.
[42]Saleeby: Philippine Jour. Sc., 1915, pp. 101, 117.
[43]Mathews: Physiol. Chem., p. 715.
[44]Vedder and Clark: Philippine Jour. Sc., 1912, 433; also Jour. Am. Med. Assn., 1916, 1495.
[45]Hehir, P.: Indian Jour. Med. Research, 1919, p. 46.
[46]Highet: Philippine Jour. Sc., 1910, Sec. B., p. 142.
[47]Steenbook, H., and Bontwell, P. W.: Jour. Biochem., Feb., 1920, p. 163.
[48]Williams, R. R.: Read before the Pan-Amer. Sc. Congress, Jan., 1916.
[49]McCarrison: Indian Jour. Med. Research, 1919, vi, No. 3, p. 275.
[50]Anrep, G. V., and Drummond, J. C.: Jour. Physiol., 1921, liv, 349.

THE EFFECTS OF THYROID, THYROXIN AND OTHER IODINE COMPOUNDS UPON THE ACETONITRILE TESTS*

By Masataro Miura, Tokyo, Japan

THE controversy over the question whether the thyroid exerts its influence in the animal body by secreting a substance which acts positively in certain processes of metabolism or whether its function is to neutralize poisons, led Hunt[1] (1905) to study the value of the thyroid in increasing the resistance of mice to poisoning with acetonitrile. He found that in mice which had received desiccated thyroid with the diet, the resistance of this poison was remarkably increased. The effect of the thyroid in some of his experiments was proportional to its iodine content, Hunt and Seidell[2] (1910). As a result of this work, however, Hunt went so far as to say that this protective action of the thyroid is a specific physiologic test for this tissue, more delicate than any known chemical test. The results were confirmed by Trendelenberg[3] (1910). On the other hand, the work of Carlson and Woelfel[4] (1910), Olds[5] (1910) and Lussky[6] (1912) seems to refute the claims of Hunt.

Although the active thyroids afforded protection against the poison in proportion to the amount of iodine contained, experiments of Hunt and Seidell[2] (1910) with a number of iodine compounds indicated that the protective action was not a function of the iodine content alone. Out of a large number of experiments, however, Hunt fed thyroid in equi-iodine dosage in only one case.

It is possible that the good results with the thyroid are the result of the amino acids of the thyroid protein supplementing the rather inadequate proteins of the wheat flour of which the mouse food of earlier workers was largely composed. That lack of vitamin or salts is not a likely factor in the susceptibility of the mice to the poison has been shown by us.

It seemed of interest, therefore, to study anew the activity of iodine in different samples of thyroid and in the other iodine compounds, including Kendall's thyroxin, in connection with the acetonitrile test. In view of the fact that on the Ehrlich cake diet, Hunt's mice lost weight, it seemed of importance to use complete diets of known nutritional value in order to obviate the possibility of a supplementing action of the thyroid proteins.

EXPERIMENTAL PART

The following food was given in all of the experiments:—

Casein 31, brewery yeast 2, salt mixture† 5, starch 40, and butter fat 22 per cent.

That casein is an adequate protein has been shown by Osborne and Men-

*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Conn. Received for publication, July 5, 1921.

†Osborne and Mendel: Jour. Biol. Chem., 1918, xxxiv, 131. The lactose and potassium iodide were omitted.

del for rats and by us for mice. On the above food all of the mice gained in weight in most of the experiments. In Table I are the results of the comparison of potassium iodide and a sample of thyroid. It will be seen that when the total amount of iodine taken as thyroid is as small as 0.0014 mg., the resistance to the poisonous action of acetonitrile is increased. With twice the dose of thyroid the resistance is doubled. When an equal amount of iodine is taken as potassium iodide, however, no increased resistance whatever is observed nor does twice or three times that amount of potassium iodide have any effect.

TABLE I

	THYROID DIET A	THYROID DIET B	I-FREE DIET	IODINE DIET A	IODINE DIET B	IODINE DIET C
Per cent iodine in substance	0.11	0.11				
Iodine in 100 gm. food (mg.)	0.055	0.110	0	0.055	0.110	0.264
Ave. daily food intake of each mouse (gm.)	2.6	2.2	2.8	2.6	2.9	2.1
Ave. daily thyroid intake by each mouse (mg.)	1.3	2.2				
Ave. daily iodine intake by each mouse (mg.)	0.0014	0.0024		0.0014	0.0032	0.0055
Number of mice	8	8	5	8	8	8
Duration of feeding (days)	9	9	9	9	9	9
Ave. change in body weight (gm.)	14.3-17.5	14.2-16.2	17.5-19.0	15.4-17.9	15.0-17.7	16.5-19.8
Minimal lethal dose acetonitrile (mg.)	1.92	3.30	0.51	0.45	0.50	0.70

In order to study the activity of the iodine in various samples of thyroid from various species of animals and to compare it with the iodine in other compounds, the following materials were used:

	Per cent Iodine
(1) Thyroid, normal, hog, summer	0.43
(2) Thyroid, normal, sheep, winter	0.06
(3) Thyroid, normal, beef, winter	0.08
(4) Thyroid, enlarged, beef, fetal (6-9 mo.)	0.06
(5) Thyroid, normal, beef, fetal (6-9 mo.)	0.28
(6) Thyroid	0.11
(7) Di-iodotyrosine	58.66
(8) Thyroxin	65.1

Thyroids (1) to (5) were obtained through the courtesy of Dr. Fenger, Armour Laboratories, and the thyroxin was kindly furnished by Dr. Kendall of the Mayo Clinic.

These materials were dried to constant weight in vacuo at room temperature in order to avoid any possible change in the physiological activity due to heat. They were then carefully triturated and incorporated in the standard diet. The di-iodotyrosine and thyroxin were mixed with starch in 0.4 per cent mixture and then used in the diet.

With di-iodotyrosine Strouse and Voegtlin[7] (1909) observed no thyroid-like effect on the nitrogen metabolism or on the blood pressure of normal dogs nor any favorable effect on the condition of myxedematous and cretinous animals. Morse[8] (1914), however, reported a positive Gudernatch test on tad-

TABLE II

	CONTROL	THYROID (1)	THYROID (2)	THYROID (3)	THYROID (4)	THYROID (5)	THYROID (6)	DI-IODOTYROSINE	THYROXIN
Per cent iodine in substance	0	0.43	0.06	0.08	0.06	0.28	0.11	58.7	65.1
Iodine in 100 gm. food (mg.)		0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Ave. daily food intake by each mouse (gm.)	2.5	2.2	2.2	2.3	1.9	2.0	2.1	1.7	2.0
Ave. daily intake of substance by each mouse (mg.)		0.85	6.05	4.75	5.23	1.18	3.15	0.003	0.005
Ave. daily intake iodine by each mouse (mg.)		0.0036	0.0036	0.0038	0.0031	0.0033	0.0035	0.0028	0.0033
Number of mice	8	8	8	8	8	8	8	8	8
Duration of feeding (days)	11	11	11	11	11	11	11	11	11
Ave. change in body weight (gm.)	15.7-18.2	14.5-14.9	14.7-15.5	14.9-14.7	15.5-17.0	15.3-16.9	16.0-16.4	15.7-17.8	15.6-16.9
Minimal lethal dose acetonitrile (mg.)	0.40	2.15	1.10	1.70	0.40	0.32	1.35	0.15	0.53

poles with this material. Thyroxin, the crystalline material isolated from the thyroid by Kendall[9] (1919), seems to embody all of the distinctive physiologic properties of the thyroid. He has shown that thyroxin cures myxedema and cretinism.

TABLE III

	CONTROL	DI-IODOTYROSINE	THYROXIN
Per cent iodine in substance		58.7	65.1
Iodine in 100 gm. food (mg.)		0.17	0.17
Ave. daily intake food by each mouse (gm.)	1.6	1.5	1.9
Ave. daily intake of substance by each mouse (mg.)		0.0042	0.0048
Ave. daily intake iodine by each mouse (mg.)		0.0025	0.0031
Number of mice	7	7	7
Duration of feeding (days)	11	11	11
Ave. change in body weight (gm.)	11.8-13.2	11.6-13.2	11.8-11.9
Minimal lethal dose acetonitrile (mg.)	0.23	0.25	0.37

In Tables II and III are given the results of the experiments on the protective action of these various substances. All were fed in such doses of the material that each mouse received an equal amount of iodine. From the table it is seen that all of the thyroids, with the exception of the fetal thyroids, gave marked protection against poisoning with acetonitrile. Thyroxin gave slight protection but di-iodotyrosine gave none. In the light of later experiments, the behavior of thyroxin in this experiment seems paradoxical. When the fetal thyroids were increased to ten times the amount the protection was increased but not to the extent afforded by the mature thyroids (Tables IV and V). Increasing the amount of di-iodotyrosine, however, had no effect, the mice receiving the increased dose having no more resistance than the controls. In this series of experiments (Table IV) it is noted that increased thyroxin failed to increase the resistance to acetonitrile, which seemed to be the case with the most active thyroid when they were both compared in experiments reported in Table VI. The case of thyroxin calls for special attention.

In Table IV are given the results showing the effect of feeding three different doses of thyroxin. Here we see that, with the largest dose, the protection against acetonitrile is smaller and there is a greater loss of weight. This led to a repeated comparison of the most active thyroid and thyroxin both being fed in doses of 0.003 mg. iodine per mouse and in three times that amount. In this series of experiments (Table VI) thyroxin compares favorably with the most active thyroid. When milk was added to the diet to prevent the loss in weight, if possible, the animals fed thyroxin not only stopped losing weight but gained more than the thyroid-fed animals. These experiments (Tables IV and VI) also show what a remarkable effect thyroxin has on the metabolism in the organism, for when thyroxin was fed in quantity approaching that of the active thyroid, the metabolism of the animal was speeded up to the point where it lost weight and this loss of weight was roughly proportional to the amount of thyroxin fed. The failure of thyroxin to protect the animals in earlier experiments (Tables II and III) may be due to the low resistance of this lot of animals to the relatively large dose of thyroxin, they being fed sixty times more than the therapeutic dose for man.

TABLE IV

	CONTROL	THYROID (4)	THYROID (5)	DI-IODOTYROSINE	DI-IODOTYROSINE	THYROXIN	THYROXIN	THYROXIN	IODINE FOOD (KI)	UNDERFEEDING
Per cent iodine in substance		0.06	0.28	58.7	58.7	65.1	65.1	65.1	76.4	
Iodine in 100 gm. food (mg.)		1.7	1.7	0.17	0.83	0.17	0.49	0.99	0.99	
Ave. daily food intake of each mouse (gm.)	1.9	2.0	2.4	1.9	1.9	1.9	1.5	1.6	1.8	1.6
Ave. daily intake of substance by each mouse (mg.)		55.0	13.8	0.0053	0.026	0.0041	0.011	0.024	0.023	
Ave. daily iodine intake by each mouse (mg.)		0.033	0.039	0.0031	0.015	0.003	0.007	0.015	0.017	
Number of mice	8	8	8	8	8	8	8	8	5	
Duration of feeding (days)	11	11	11	11	11	11	11	11	11	
Ave. change in body weight (gm.)	17.9-18.7	18.3-15.8	18.7-17.3	18.5-17.9	18.7-17.7	18.8-17.5	18.4-15.4	18.4-14.8	18.2-18.3	19.9-18.7
Minimal lethal dose acetonitrile (mg.)	0.34	0.91	2.34	0.29	0.21	0.624	0.57	0.56	0.34	0.37

Before further discussion, it is important that we have a summary of the experimental facts before us. The protective action of the thyroid is not a function of the iodine content alone for potassium iodide or di-iodotyrosine when given by mouth does not protect mice from poisoning with acetonitrile. Thyroids vary greatly among themselves in their ability to give resistance to acetonitrile poisoning—the fetal glands being least valuable in this respect. Although Hunt reported that the active thyroids gave resistance to acetonitrile in proportion to their iodine content, the most active thyroid tried by us did not behave in this way. Thyroxin, in the smallest dose used, may be as potent as the most active thyroid; in increasingly large doses it not only lowers the resistance of the animal to acetonitrile but also causes loss in weight.

TABLE V

	CONTROL	DI-IODOTYROSINE	THYROID (5)	THYROXIN
Per cent iodine in substance		58.7	0.28	65.1
Iodine in 100 gm. food (mg.)		0.825	1.7	0.17
Ave. daily food intake by each mouse (gm.)	2.4	2.3	2.6	2.6
Ave. daily intake of substance by each mouse (mg.)		0.032	14.99	0.0065
Ave. daily intake iodine by each mouse (mg.)		0.019	0.04	0.004
Number of mice	5	4	8	6
Duration of feeding (days)	7	7	7	7
Ave. change in body weight (gm.)	20.2-20.5	18.6-18.7	17.2-16.7	19.3-18.4
Minimal lethal dose acetonitrile (mg.)	0.60	0.24	4.8	4.0

TABLE VI

	CONTROL	THYROID (1)	THYROID (1)	THROXIN	THROXIN	THROXIN + MILK
Per cent iodine in substance		0.43	0.43	65.1	65.1	65.1
Iodine in 100 gm. food (mg.)		0.17	0.495	0.17	0.495	0.17
Ave. daily food intake per mouse (gm.)	1.81	2.09	2.06	2.08	1.79	1.7
Ave. daily intake of substance by each mouse (mg.)		0.80	2.37	0.005	0.013	0.004
Ave. daily intake of iodine by by each mouse (mg.)		0.003	0.01	0.003	0.009	0.003
Number of mice	7	8	8	8	8	6
Duration of feeding (days)	10	10	10	10	10	10
Ave. change of body weight (gm.)	16.5-18.9	16.3-17.1	16.4-17.4	16.5-17.6	16.1-14.7	15.1-16.6
Minimal lethal dose acetonitrile (mg.)	0.45	6.4	6.0	5.6	5.2	6.0

Why do the various thyroids differ in their ability to protect mice against acetonitrile? It is not due to the iodine content for they were all fed in equi-iodine doses. It is probably not due to a species difference, for the beef thyroids differed among themselves. It may be that in the preparation the material was rendered inert,—a possibility but not a probability. Or it may be that the iodine is combined in varying ratios of active and inactive forms. In the light of Kendall's[10] (1915) recent work the latter hypothesis seems to be applicable. According to him all of the iodine of the thyroid can be divided by alkaline hydrolysis into two forms: the "A" form, comprising one-half of all the iodine, insoluble in acid, curing myxedema and cretinism and from

which thyroxin was isolated, and the "B" form, comprising one-half of the iodine, acid soluble but almost inert physiologically. In another place Kendall[11] (1917) states that the "A" form may vary from 2 to 3 per cent to 40 to 50 per cent which might help explain some of our results.

TABLE VII

	CONTROL	THYROID (1)	THYROXIN
Per cent iodine in substance	0	0.43	65.1
Iodine in 100 gm. food (mg.)		0.17	0.06
Ave. daily food intake of each mouse (gm.)	1.86	1.8	1.8
Ave. daily intake of substance by each mouse (mg.)		0.69	0.002
Ave. daily iodine intake by each mouse (mg.)		0.003	0.00099
Number of mice	6	6	6
Duration of feeding (days)	11	11	11
Ave. change of body weight (gm.)	16.3-18.0	16.1-17.3	16.1-16.8
Minimal lethal dose acetonitrile (mg.)	0.48	2.08	3.20

If the ratio of "A" iodine to "B" is 1:1 in the thyroids it may be that we have been feeding thyroxin in too strong a concentration, since it corresponds closely to the "A" form and we fed our material in equi-iodine amounts. Therefore the experiments recorded in Table VII were performed, with the result that when thyroxin was fed in one-third the amount of the most active thyroid preparation (based on the iodine content) the protection against acetonitrile was greater than that given by the thyroid. This seems to point to the possibility that it is the "A" iodine in the thyroid which is concerned in producing the resistance to acetonitrile and that thyroxin, which is obtained from this fraction, contains the protective substance in very high concentration.

Kendall[12] (1918) states that the thyroid, through the action of its hormone, determines how much energy a given cell can produce on stimulation from without or within. In other words, the thyroid hormone is an important link in physiologic oxidation. He also states that the imino group is the one in thyroxin which is concerned in the metabolic effects and that the iodine only serves to make the imino group more reactive. That the iodine has no effect upon metabolic rate has been shown by Kendall[13] (1919) in experiments in which the imino group of the thyroxin was substituted, but he was able to influence the metamorphosis of frogs with this material the same as when the imino group was unchanged. Which group in thyroxin is responsible for the protective action against acetonitrile? The fact that thyroids vary in their protective action so markedly when fed in equi-iodine amounts, the fact that iodine-free thyreoglobulin (Hunt[14] 1907) produces a similar effect to that of iodine-containing thyroids, the minute amount required compared to that of other iodine-containing compounds all indicate that the protective action of the thyroid depends upon the metabolic effect of its active principle rather than upon the iodine contained therein.

On the other hand, it is known that acetonitrile produces its bad effect through its oxidation to hydrocyanic acid. If, now, the beneficial effect of thyroxin is due to its tonic effect on the normal cell which ultimately means facilitated oxidation, it seems difficult to understand the mechanism whereby a

substance promoting oxidation can protect an organism from a reaction depending on oxidation for its harmfulness. Although the present investigation has not disclosed the ultimate mechanism of thyroid action, it indicates that thyroxin is indeed an active principle of the thyroid which increases the resistance of mice to acetonitrile.

SUMMARY

(1) Desiccated thyroid fed to mice protects them against poisoning with acetonitrile. The thyroid seems to be efficient in proportion to the amount of iodine contained.

(2) Potassium iodide and di-iodotyrosine gave no protection against acetonitrile in whatever quantity fed.

(3) Thyroxin when fed in such an amount that it furnished one-third the iodine provided by the most active thyroid fed, in some cases gave a greater degree of protection. Increasing the amount of thyroxin, decreased the protection against acetonitrile and caused loss of weight of the animals.

The author wishes to express his gratitude to Professor Lafayette B. Mendel for his helpful advice and criticism.

REFERENCES

1Hunt: Jour. Biol. Chem., 1905, i, 39.
2Hunt and Siedell: Jour. Pharm, and Exper. Therap., 1910, ii, 15.
3Trendelenberg: Biochem. Ztschr., 1910, xxix, 396.
4Carlson and Woelfel: Am. Jour. Physiol., 1910, xxvi, 32.
5Olds: Am. Jour. Physiol., 1910, xxvi, 354.
6Lussky: Am. Jour. Physiol., 1912, xxx, 63.
7Strouse and Voegtlin: Jour. Pharm. and Exper. Therap., 1909-10, i, 123.
8Morse: Jour. Biol. Chem. 1914, xix, 421.
9Kendall: Jour. Biol. Chem., 1919, xxxix, 125.
10Kendall: Jour. Biol. Chem. 1915, xx, 501.
11Kendall: Endocrinology, 1917, i, 167.
12Kendall: Jour. Am. Med. Assn., 1918, lxxi, 871.
13Kendall: Proc. Am. Phys. Soc., April, 1919, 136. Am. Jour. Physiol., 1919, xlix.
14Hunt: Jour. Am. Med. Assn., 1907, xlix, 1323.

LABORATORY METHODS

AN ADAPTATION OF THE FOLIN AND HU BLOOD SUGAR METHOD APPLICABLE TO SMALL AMOUNTS OF BLOOD*

A Comparison of the Blood Sugar Content of Venous and Capillary Blood

By Emil J. Baumann, Ph.D., and Rae L. Isaacson, New York City

IT is very desirable and in fact often necessary to estimate the blood sugar by some procedure requiring only the small amounts of blood that may be obtained from pricking the finger. Various micro methods of this kind have been used, the Bang,[1] adaptations of the picric acid method of Lewis and Benedict[2] and Dehn and Hartman,[3] and the MacLean,[4] being among those more commonly employed. The Bang procedure while long and most exacting, is so apt to give erroneous results that analyses must be done in triplicate. The picric acid method of Epstein[5] has often been found to yield results considerably higher than those obtained by other procedures and we have had similar experiences with it. Like all colorimetric methods in which the color measured is the resultant of two or more colors, in this case sodium picrate and sodium picramate, serious errors are often introduced as shown by Falk and Noyes.[6] The work of the Harriman Research Laboratory investigators has raised a serious objection on both theoretical and practical grounds, to the use of any picric acid reduction method, where another procedure is available. The MacLean method seems to have yielded excellent results though it is quite long and great care is said to be necessary.

When the systematic methods of blood analyses of Folin and Hu[7] were published, they were tried out in this laboratory, and after thorough testing, found to be very satisfactory, and adopted as our standard procedures. With a view to keeping our methods limited to trustworthy ones, and as few as possible, we endeavored to adopt the blood sugar method of Folin and Hu for an amount of blood that could be obtained by pricking the finger. This we have succeeded in doing in a very satisfactory way, using 0.4 c.c. of blood. The adaptation is comparatively simple and rapid and is free from the objections that have just been pointed out. It has been in use here and at other laboratories for the past two years. The procedure is as follows:

Ordinary one c.c. serologic pipettes, graduated to the tip, in 0.01 c.c. are cut off at about the 0.6 c.c. mark and calibrated to the 0.4 c.c. mark. The pipette is connected with a rubber tube, on the end of which a small glass mouthpiece is placed. Blood is obtained, usually from the fourth, occasionally from the middle finger of the left hand by pricking with a Hagedorn needle.

*From the Laboratories, Montefiore Hospital, New York.
Received for publication, July 22, 1921.

Gentle pressure is applied if necessary, not too near the wound, until a large drop of blood has collected. This is carefully drawn up the pipette, in the beginning mainly by capillarity, but after about 0.2 c.c. of blood has been obtained, very gentle suction must be applied to the glass mouthpiece. The surface of the skin is kept quite dry with sterile sponges to keep the blood in a deep round drop; if the skin is not dry the blood will spread.

After 0.4 c.c. of blood has been obtained, it is immediately discharged into a 5 c.c. tube graduated in 0.1 c.c. containing about one c.c. of distilled water. The corpuscles settle to the bottom of the tube and a clot will form unless the tube is gently rotated. Frothing should be avoided. The pipette is then quickly washed with water several times, the washings added to the tube and the total volume brought to 3.2 c.c. Four tenths of a c.c. of ten per cent sodium tungstate and an equal volume of 0.7N sulphuric acid are added successively, the tube is closed with a cork or rubber stopper, shaken vigorously and allowed to stand ten or fifteen minutes. Then centrifuge in the same tubes for ten or fifteen minutes at a high speed. Filter the supernatent liquid through a very small filter (4¼ cm.) into a 15 c.c. or 30 c.c. beaker.

Two c.c. of the filtrate are pipetted into a Folin sugar tube and Folin and Hu's procedure followed exactly as in the macro method. That is, 2 c.c. of standard sugar solutions, equivalent to 100 and 200 mgs.* of glucose per 100 c.c. respectively, are pipetted with Folin-Ostwald pipettes into similar sugar tubes. To both the unknown and the standards, 2 c.c. of the faintly alkaline copper tartrate solution are added and all heated simultaneously in a boiling water-bath for exactly six minutes. The tubes are then quickly plunged into cold water and kept there for two minutes, after which two c.c. of the molybdate phosphate solution are added to each. The tubes are shaken vigorously, allowed to stand about five minutes, made up to 25 c.c. and matched. Not more than four tubes should be heated at once unless more than one person can be at hand so that the molybdate phosphate solution can be added nearly simultaneously to all the tubes. The color remains practically unchanged for more than one hour.†

A comparison of results obtained by the micro and macro methods is presented in Table I.

---

*Containing 0.2 and 0.4 mg. of glucose respectively.

† For convenience, the preparation of reagents as described by Folin and Hu is given:

Ten per cent Sodium Tungstate.—Purchased from the Vanadium Corporation of America, Primos, Delaware Co., Pa. Labelled Primos Brand and C. P.

Alkaline Copper Tartrate Solution.—Dissolve 40 gms. of pure anhydrous sodium carbonate in about 400 c.c. of water and transfer to a liter flask. Add 7.5 gms. of tartaric acid and when the latter has dissolved add 45 gms. of crystallized copper sulphate. Mix and dilute to one liter. If the reagents used are impure a sediment of cuprous oxide may separate in the course of one or two weeks. Should this occur, remove the clear supernatant fluid with a siphon or filter through a good quality filter paper. To test for the absence of cuprous copper, in the solution, transfer 2 c.c. to a test tube and add 2 c.c. of the molybdate phosphate solution; the deep blue color of the copper solution should almost completely vanish.

Standard Sugar Solutions.—Three standard sugar solutions should be on hand: (1) a one per cent pure glucose solution, which is kept as a stock solution, preserved with a thin layer of toluene or xylene; (2) a solution containing 1mg. of glucose per 10 c.c. (5 c.c. of stock solution diluted to 500 c.c.); (3) a solution containing 2 mg. of sugar per 10 c.c. (5 c.c. of stock solution diluted to 250 c.c.). The diluted solutions are also preserved with a little toluene or xylene. The diluted solutions will not keep for much more than a month in warm weather, but the stock solution should keep indefinitely.

Molybdate Phosphate Solution.—Transfer to a liter beaker 35 gms. of molybdic acid and 5 gms. of sodium tungstate. Add 200 c.c. of 10 per cent sodium hydroxide and 200 c.c. of water. Boil vigorously for 20 or 40 minutes so as to remove nearly the whole of the ammonia present in the molybdic acid. Cool, dilute to about 350 c.c. and add 125 c.c. of concentrated (85 per cent) phosphoric acid. Dilute to 500 c.c.

TABLE I

	COMPARISON OF MACRO AND MICRO METHODS		
	MACRO	MICRO	REMARKS
Mrs. P.	174	174	Diabetes
A. B.	105	105	
A. 1.	150	155	After lunch
Mrs. H.	320	323	Diabetic coma
Dog 1	100	96	
Dog 2	132	130	During absorption
Dog 2	241	239	1 1/2 hrs. after glucose administration
Sheep 1	75	77	

In Table II the results of analyses of blood, drawn successively from the median cephalic vein and finger tip are given. After the blood was drawn from the arm, it was immediately taken to the laboratory and precipitated according to the method of Folin and Hu. As soon as blood had been drawn from the vein, specimens were obtained from the finger tip and analyzed by the method just described.

TABLE II

COMPARISON OF BLOOD SUGAR CONTENT OF VENOUS AND CAPILLARY BLOOD		
	VENOUS BLOOD MG. PER 100 C.C.	CAPILLARY BLOOD MG. PER 100 C.C.
X. B.	100	107
Mrs. P.	129	139
M.	98	108
H. S.	104	101
O. L.	92	96
? W.	120	123
? K.	101	103
B. J.	246	247
E. J.	386	390
? R.	94	99
D. C.	135	142
D. C.	117	121
L. R.	111	106
? R.	119	110
L.	92	96
Dr. A.	100	103
W.	128	128
W. D.	103	102
M. S.	106	102
S. S.	94	95
D. C.	114	110
	105	95
S. W.	90	95
H. P.	139	136
M. G.	132	134
F. I.	106	107

As might be expected on theoretical grounds, the capillary blood usually contained very slightly more reducing substances than venous blood. However, these variations may have been due to a little glycolysis of the venous blood. Occasionally on account of the distance from the wards as much as ten or fifteen minutes elapsed between the time the blood was drawn and the proteins precipitated, while in the case of the micro determinations, the precipitations were made at once.

Glucose solutions added to blood were in every instance recovered quantitatively by the above described micro method.

BIBLIOGRAPHY

[1]Bang, I.: Der Blutzucker, 1913.
Bang, I.: Über die Mikromethode der Blutzuckerbestimmung, Biochem. Ztschr., 1913, lvii, 300-313.
[2]Lewis, R. C. and Benedict, S. R.: Estimation of Sugar in Blood, Jour. Biol. Chem., 1915, xx, 61-72.
[3]Dehn, Wm. M. and Hartman, F. A.: A Picrate Colorimetric Method for the Estimation of Carbohydrates, Jour. Am. Chem. Soc., 1914, xxxvi, 403-409.
[4]Gardener, A. D. and MacLean, H.: A Method for the Estimation of Sugar in Blood with Observations on some Modern Methods, Biochem. Jour., 1914, viii, 391-403.
[5]Epstein, A. A.: An Accurate Micromethod of Estimating Sugar in the Blood. Jour. Am. Med. Assn., 1914, lxiii, 1667-8.
[6]Falk, K. G. and Noyes, H. M.: Some Observations on Colorimetric Estimations with Solutions Containing two Colored Substances. Jour. Biol. Chem., 1920, xlii, 109-130.
[7]Folin, O., and Hu, H.: A System of Blood Analyses, Jour. Biol. Chem., 1919, xxxviii, 81-110.
Folin, O., and Hu, H.: Improved Method for Determination of Sugar, Jour. Biol. Chem., 1920, xli, 367-374.

A NOTE ON THE STERILIZATION OF SURGICAL INSTRUMENTS*

By Everett S. Sanderson, New Haven, Conn.

In a brief article written by Dr. Hibbert W. Hill, *Journal of Applied Microscopy*, Vol. iii, No. 8, the author describes the following method for sterilizing instruments during Bacteriological Autopsy work: The blades, knives, scissors, forceps, etc., are dipped into methyl alcohol and then quickly passed close enough to a bunsen flame to ignite the alcohol. The alcohol burns off instantly, leaving a dry, sterile surface free from any disinfectant.

The advantage over direct flaming lies in the tendency of the latter process to destroy the temper of the instrument. The advantages over the use of disinfecting solutions, as carbolic acid, etc., are: (1) No disinfectant is introduced into the incised tissue by the knife; (2) Sterilization is instantaneous and complete, whereas, disinfectant solutions require some appreciable time to kill the common pathogenic forms, and a considerable time to kill spores; (3) If the knife has already been used and sterilization is required, the adherent blood or tissue is readily cleared and rendered sterile, while a disinfectant solution requires time for penetration. Experimentally, also, it has been found that anthrax spores smeared upon a knife blade failed to develop when transferred to media after the blade had been flamed once in alcohol, as described above.

It appeared to the writer that the "time" in which it took to burn off alcohol† from a smooth surface was really insufficient to completely sterilize it, especially if organisms were present in the free spore stage. Hence, it seemed desirable to further investigate this method of sterilization, and if it

*From the Laboratory of Bacteriology, Sheffield Scientific School, Yale University.
Received for publication, July 5, 1921.
†Ordinary 95 per cent alcohol.

proved inefficient to determine whether or not any other inflammable fluid will accomplish the sought-for end. With these two aims in view, numerous experiments were carried on, the results of which constitute the basis of this article.

It seemed logical to begin with organisms of the nonspore-forming type, and consequently, the following representatives were chosen from the stock cultures of this laboratory: *Bact. coli, Ps. pyocyanea, Staph. aureus, Staph. albus, Strep. pyogenes,* and *Proteus vulgaris.*

For lack of sufficient scalpels to do this work on a large scale, glass rods, as advocated by Dr. Hill for disinfection experiments, were also employed. Yet, insofar as the present results indicate, there was no difference whether a glass or metal surface was employed.

Sterile glass rods were dipped into saline emulsions of the above-mentioned organisms and then placed each into a sterile test tube and allowed to dry. This accomplished, the rods were dipped, one by one, into methyl alcohol and passed close enough to a flame to ignite them. Each in turn was then rubbed over the surface of an agar slant, withdrawn, and quickly planted into a tube of plain bouillon. In all cases, rods containing the emulsion, but unburned, were run as controls. Agar and broth tubes were incubated at 37° C. and results noted at the end of 24 hours.

With these nonspore-bearing organisms no growth was ever obtained, either in broth or on agar, with rods which had been burned off, even after several days of reincubation. Controls always yielded positive growth.

Convinced that this method of sterilization, at least when employed on clean surfaces, is effective for nonspore-forming organisms, attention was next directed to members of the spore-bearing group. The following representatives were used from among the laboratory stock cultures: *B. cereus, B. anthracis, B. tetani, B. botulinus, B. sporogenes, Reading Bacillus.* Since this group comprises both aerobes and anaerobes, slight variations in the procedure were necessary. With *B. cereus* and *B. anthracis,* emulsions were made in sterile saline solution from old agar cultures which upon microscopic examination indicated a preponderance of free spores. As before, sterile glass rods were dipped into the emulsions and allowed to dry in sterile test tubes. The rods in turn were immersed in methyl alcohol, ignited, drawn over agar slanted in tubes, planted finally into plain broth, and incubated at 37° C. After 24 hours abundant growth never failed in the numerous times that these experiments were repeated. All controls gave positive growth also.

As for the anaerobes, old cultures growing in the egg-meat medium employed by Rettger, were used, the presence of spores having first been confirmed by a microscopical examination of each culture. Sterile glass rods were dipped into these cultures and then replaced within sterile test tubes and time given to allow the greasy material to firmly adhere to the rods. This accomplished, each in turn was immersed in methyl alcohol, ignited, planted into a tube of egg-meat and incubated at 34° C. In no instance did characteristic, abundant growth fail to take place either in tubes from rods which had been burned off, or in the controls which had not been so treated.

From the foregoing results, it seems logical to conclude that the short interval of time consumed in the burning off of a glass rod with methyl alcohol is insufficient to sterilize the surface when spores of the organisms here employed are present.

Having found that methyl alcohol in itself was inadequate, the question arose as to whether there was any liquid, which, when applied to a surface and ignited, would completely sterilize it, and, in case of an instrument, leave a clean and uninjured surface. To determine this, mixtures of different fluids with both methyl and ethyl alcohol were tried. They were as follows:

1. Methyl alcohol plus formalin (3:1).
2. Methyl alcohol plus kerosene (3:1).
3. Methyl alcohol plus benzol (3:1).
4. Ethyl alcohol plus formalin (3:1).
5. Ethyl alcohol plus 5 per cent phenol.

As in previous experiments, the infected rods or scalpels were dipped into these solutions, ignited and planted into media; but with the exception of the mixtures Nos. 1 and 4, the results proved to be no better than those obtained when alcohol alone was used. Furthermore, in the case of kerosene and benzol, the burnt surface was always coated with a carbon deposit. With the alcohol formalin mixtures, no growths were obtained, and so the methyl alcohol formalin solution was selected to be employed in further investigations. Using the spores of *B. anthracis* and *B. cereus*, some thirty-five tests were made, and from these growth developed in none but two (two colonies on an agar slant in one instance, and four in the other). When spore material of the previously mentioned anaerobes was tried, growth in the egg-meat medium resulted in all instances, although as compared with the controls, it required two to three days longer before turbidity or liquifaction set in. In all probability the reason for this failure to sterilize was due to the fact that the oil or grease which is deposited upon the rods in the process of immersing them in cultures of egg-meat medium interfered with the even distribution of the alcohol formalin mixture over the surface.

It was next desired to ascertain whether or not methyl alcohol plus formalin would completely sterilize when blood is present. Hence, blood was drawn from a rabbit's ear into a sterile Petri dish and mixed with a suspension containing anthrax spores. Glass rods and scalpels were smeared with this material and set aside in sterile test tubes to dry for three hours. At the end of this period, each rod was rubbed onto an agar slant as a control, and then burned off with the alcohol formalin mixture, drawn over another agar slant and finally planted into a tube of plain broth. The scalpels were drawn over one-half the surface of a divided agar plate before burning, and over the remaining half after burning (the former being the control). All controls showed growth at the end of 24 hours, while transfers from the rods and scalpels after burning developed no growth whatever after five days' incubation. Parallel to this series, blood-treated and infected rods were burned off with methyl and ethyl alcohol, the subsequent procedure being similar to that above. In all cases, growth was present at the end of 24 hours.

Having observed that the burning off of a surface with alcohol alone had no sterilizing action when spores were present, it was naturally assumed that the flame was not hot enough to accomplish the desired result in the limited time required for the alcohol to burn. In order to obtain some idea as to the intensity of the heat produced, the bulb and lower portion of an ordinary chemical thermometer were immersed in alcohol, ignited, and the maximum rise of the mercury noted. This was repeated several times, the mercury being brought back to room temperature before the next trial was attempted. For methyl alcohol, the mercury stood on an average at 69° C.; ethyl alcohol 60° C.; methyl alcohol formalin mixture 133° C. The flame in the last-mentioned test might not have been actually any more intense than the others, but it burned for a longer period of time, which after all is a very important factor in all disinfection work. In view of the fact that some of the anthrax spores used in the above experiments were able to withstand boiling for several minutes, it does not seem possible that even a temperature reaching a maximum of 133° C. in a few seconds could in itself produce sterility. Hence, it seems logical to conclude that the results obtained with the alcohol formalin mixture were due to the chemical action of formalin in conjunction with heat.

SUMMARY AND CONCLUSIONS

The results set forth in this paper may be summarized as follows:

(1) No growth was obtained when either glass rods or scalpels on which nonspore-forming organisms were present were burned off with methyl alcohol.

(2) Glass rods and scalpels could not be sterilized by burning them off with methyl alcohol when spores of *B. anthracis, B. cereus, B. botulinus, B. tetani,* etc., were present.

(3) The burning off of glass rods and scalpels with a methyl alcohol-formalin mixture (3:1) gave excellent results except in the case of the anaerobes when grease and oil were present.

(4) When absolute sterility is desired, the burning off of a surface with an inflammable fluid cannot *always* be relied upon.

A MICRO-MODIFICATION OF THE METHOD OF BENEDICT FOR THE QUANTITATIVE DETERMINATION OF REDUCING SUGAR IN URINE*

By Millard Smith

OF the various methods for the quantitative determination of reducing sugar in urine, doubtless none is in more general use than that of Benedict.[1] This method, due to its simplicity, is unusually adapted to routine clinical work and perhaps in the hands of the technician gives results which are more accurate than would other methods which might claim on theoretical grounds the advantage of greater accuracy in the hands of the experienced chemist.

However there is coming into quite common use a modification of the Benedict method which is based upon a reasonable assumption but which in its application does not conform to the requirements therein demanded. Moreover there has apparently been no attempt to verify the correctness of the modification and unfortunately this has led to the use of a method which has an appreciable degree of error.

The argument usually given in defense of the use of the modification is that it considerably reduces the cost of the determination and at the same time greatly simplifies it. In connection with the investigation of the accuracy of the procedure the writer has therefore attempted to so modify the original method that these advantages may be retained and at the same time to avoid the error which is encountered in the present modification. It has been possible to do this satisfactorily.

The modification which is being used at present can be ascribed to no one individual for it seems to have been in use independently in several laboratories and was merely a natural deduction in the interest of economy and efficiency. It consists in the use of from one to two-fifths of the original amount of Benedict's solution specified for a determination. The amount of carbonate is correspondingly reduced but the original volume is retained by the addition of 15 to 20 c.c. of distilled water. The titration is then carried out as usual in a porcelain dish.

It might seem that such a procedure would be permissible but this slight modification leads to a variable error of from 15 to 30 per cent. The explanation for the error lies in the disturbed alkalinity of the titrating solution which is caused by the dilution of the reagent with water. Even the use of the same amount of carbonate as in the original determination does not remedy the error. The buffer action of the citrate in definite concentration is evidently necessary for the proper reaction. Even with the original method a similar degree of error is encountered in the determination of a urine of low sugar

*From the Clinical Laboratory of Dr. Elliott P. Joslin at the New England Deaconess Hospital, Boston.
Received for publication, September 9, 1921.

content, i.e. 0.2 per cent, if care is not taken to keep the volume of the titrating solution constant. This necessitates the evaporation during the titration of the entire amount of urine added, which amounts to 25 c.c. Such a determination is time-consuming and laborious. The endpoint is very difficult and unsatisfactory to read.

Another difficulty often encountered with the modified method is a precipitation of the red oxide of copper which obscures the endpoint. This is due to the dilution of the potassium sulphocyanate and potassium ferrocyanide. These two reagents are needed in good concentration to hold the red oxide in solution. However, as pointed out by Benedict,[2] this could be avoided by the use of more ferrocyanide in the standard solution.

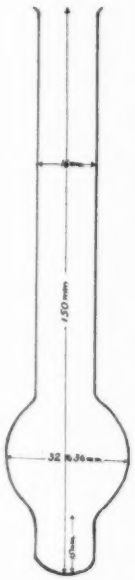

Fig. 1.

There seems to be no reason why correct results could not be obtained if the original assumption (that proportionate parts of the reagents should do as well as the full amount) was strictly adhered to. This has been done and the result has been very gratifying. The difficulty of the determination of very weak (0.2 per cent) sugar urines has largely been overcome, the endpoint is considerably sharper and the time for a determination has been reduced. This has been secured by the use of 1 c.c. of the original Benedict's quantitative solution without the addition of water but with a proportionate reduction of carbonate. The determination is carried out in a special test tube (See Fig. 1) rather than in a porcelain dish. With the use of such a small quantity of Benedict's solution evaporation would be too rapid from a dish to keep the volume during the titration constant. In the tube it is much easier to control evaporation, for with moderate boiling it is almost nil, while with vigorous boiling it is fairly rapid. The detection of the endpoint is also greatly facilitated by the use of a glass tube. The bulb is necessary to aid even boiling and to prevent too rapid evaporation and foaming.

REAGENTS AND APPARATUS

1. Benedict's Quantitative Sugar Reagent.

Copper Sulphate. 5 H_2O, C.P.	18.0 gm.
Sodium Carbonate (anhydrous)	100.0
Sodium Citrate. 5½ H_2O, C.P.	200.0
Potassium Sulphocyanate, C.P.	125.0
Potassium Ferrocyanide (5 per cent sol.)	5.0 c.c.

"With the aid of heat dissolve the carbonate, citrate and sulphocyanate in enough water to make about 800 c.c. of the mixture and filter if necessary. Dissolve the copper sulphate separately in about 100 c.c. of water and pour the solution slowly into the other liquid, with constant stirring. Add the ferrocyanide solution, cool and dilute exactly to 1000 c.c. Of the various constituents the copper sulphate only need be weighed with great exactness." The use of C. P. Citrate is very desirable as the U.S.P. grade gives a distinctly brownish tinge to the endpoint.

2. Sodium Carbonate (anhydrous).

3. Special test tube for titration.* This tube is preferably but not necessarily made from Pyrex glass. An ordinary test tube of proper size and blown as in Fig. 1 will serve the purpose, but the greater mechanical strength of Pyrex glass will soon pay for the initial cost of the tubes by cutting down their breakage.

The dimensions of the tube are as indicated in the illustration. Slight variations from these dimensions are not significant but the part of the tube below the bulb should be of such size as to contain not over 2.0 c.c. or under 1. 5 c.c.

4. Mohr pipette; capacity 0.4 c.c.† This pipette is to be made and graduated as follows. Distance between 0.00 c.c. and 0.4 c.c. to be between 21 and 22 cm. Tip for delivery to be about 3 cm. below lowest graduation and to be drawn to such a point that it will deliver a drop of water of not over 0.02 c.c. Upper end to be about 5 cm. above highest graduation. Pipette to be graduated every 0.02 c.c. but marked to read directly per cent sugar in urine. Markings to be every 0.02 c.c. from above downwards as follows: Per cent 100.00—10.00—5.00—3.33—2.50—2.00—1.65—1.43—1.25—1.11—1.00—.91—.83—.77—.67—.63—.59—.56—.53—.50—

5. Mohr pipette; capacity 2.00 c.c.† This pipette is to be made and graduated as follows: Distance between 0.00 c.c. and 2.00 c.c. to be between 20 and 22 cm. Tip for delivery to be about 3 cm. below lowest graduation and drawn to such a point that it will deliver a drop of water of not over 0.05 c.c. Upper end to be about 5 cm. above highest graduation. To be graduated only in per cent of sugar in urine with the graduations to come at the following points:

% graduation	c.c.
100.00	0.00
2.00	.10
1.00	.20
.70	.29
.60	.33
.50	.40
.40	.50
.30	.67
.20	1.00
.18	1.10
.17	1.20
.15	1.30
.14	1.40
13	1.54
.12	1.70
.11	1.82
.10	2.00

6. Ostwald pipette; capacity 1.0 c.c. accurately calibrated for blow-out delivery. If desired a 1.0 c.c. Mohr pipette calibrated to deliver between two marks may be used instead of the Ostwald.

DETERMINATION

Accurately pipette 1.0 c.c. of Benedict's solution into the test tube (which may be held in a clamp on a ring stand) and then add 0.2 to 0.7 gm. of

*These tubes may be purchased from the Emil Greiner Co., 55 Fulton St., New York City, for 50 cents (Pyrex) each.

†The Emil Greiner Co., will supply these pipettes for 80 cents each.

anhydrous sodium carbonate. A small, well dried pebble, or a piece of quartz, should also be added. (Benedict[3] suggests the use of a small piece of cotton to prevent bumping.)

Heat the mixture to boiling and add the urine from the Mohr pipette until reduction is complete as evidenced by the disappearance of the blue color. For a urine that is apt to contain 1 per cent or less sugar, the 2.0 c.c. Mohr pipette is used, and for stronger urines the 0.4 c.c. pipette. The approximate strength of the urine, with a slight amount of experience, is easily estimated from the qualitative Benedict test. It has been found that with the use of a 0.4 c.c. Mohr pipette graduated in 0.02 c.c. it is unnecessary before titration to dilute urines under 3 per cent in order to ensure sufficient accuracy. Urines over 3 per cent should be diluted to secure an accurate titration.

For *rapid* reduction of the reagent one must resort to vigorous boiling, but this may cause trouble due to too rapid evaporation and in fact it is not necessary if sufficient time is given for reduction between additions of the urine. If the solution is kept *at* the boiling point by manipulation of the flame and the urine added slowly the best results are obtained. *The tendency in the titration of sugar is to go past the endpoint. This is because the reduction does not take place as rapidly as the ordinary titrations to which one is accustomed. When nearing the endpoint the urine must be added slowly.* In urines of low sugar content the boiling should be rather vigorous at first in order to maintain a constant volume while the 1.0 to 2.0 c.c. of urine necessary to give complete reduction are being added. With a moderate amount of practice the regulation of the volume of the boiling solution becomes quite simple. A micro-Bunsen burner should be used.

CALCULATION

With the use of the pipettes described no calculation is necessary as the graduations read directly the per cent of sugar in the urine. However the use of these pipettes is not necessary and in their place a Folin 5.0 c.c. micro-burette for urine sugars may be used. The calculation is then:

$$\frac{0.02}{\text{c.c. urine}} = \text{per cent sugar in the urine.}$$

With urines which have been diluted the per cent obtained should of course be multiplied by the dilution.

DISCUSSION OF RESULTS

Table I gives a short series of determinations made by all three methods. These determinations have not been made as an attempt to indicate the greatest accuracy obtainable by either method but rather to show the accuracy to be expected when used with reasonably careful technic in the routine work of the clinical laboratory. Undoubtedly greater accuracy may be obtained by the very careful use of a Folin micro-burette and the repeating of a determination after the approximate value has been determined by a rapid preliminary titration. It will be seen in the table that the average error

TABLE I

PER CENT REDUCING SUGAR IN URINE

URINE	BENEDICT'S METHOD	WRITER'S MODIFICATION	COMMONLY USED MODIFICATION
I	.13	.13	.10
II	.17	.17	.14
III	.36	.40	.26
IV	.37	.35	.26
V	.40	.43	.29
VI	.78	.77	.54
VII	.77	.78	
VIII	1.26	1.27	1.09
IX	1.90	2.00	1.49
X	1.98	2.00	
XI	2.69	2.50	1.90
XII	3.16	3.33	2.63
XIII	5.90	5.00	4.76
	1.53	1.47	

for the writer's modification is about plus or minus 5 per cent, the average error for the modification commonly in use being always minus about 24 per cent with variations between 15 and 30 per cent. Urine XII was titrated without diluting it and it may be seen that the titration of such a concentrated urine without dilution involves considerable error.

The titration of urines containing less than 0.17 per cent sugar is very unsatisfactory by either the original method or the writer's modification. Perhaps the most constant results (although involving a positive error of 5 to 10 per cent) is to add to the tube before boiling a good sized pinch of talcum powder. The titration is then carried out as directed and the endpoint is read while the solution is boiling. It will be white or nearly so, but when the tube stands and the talcum powder settles to the bottom it will be seen that the solution is a light dirty green. It is however better to call this the endpoint than to use no talcum powder and try to titrate until the dirty green color disappears. The writer has not found this procedure necessary for urines containing over 0.2 per cent sugar.

CONCLUSION

Figures are given to prove the inaccuracy of a modification of Benedict's quantitative urine sugar method now in common use.

A modification is outlined which will give correct values and at the same time greatly cut the cost and time of the determination as well as increase the ease with which the endpoint of the titration is read and the various operations are performed.

BIBLIOGRAPHY

[1]Benedict, S. R.: The Detection and Estimation of Glucose in Urine, Jour. Am. Med. Assn., 1911, lvii, 1193.

[2]Benedict, S. R.: The Detection and Estimation of Reducing Sugars, Jour. Biol. Chem., 1907, iii, 101.

[3]Benedict, S. R.: The Detection and Estimation of Reducing Sugars, Jour. Biol. Chem., 1907, iii, 101.

The Journal of Laboratory and Clinical Medicine

VOL. VII. MARCH, 1922 No.

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.
Washington, D. C.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - CINCINNATI
HANS ZINSSER, M.D. - - - NEW YORK
PAUL G. WOOLLEY, M.D. - - DETROIT
FREDERICK P. GAY, M.D. - - BERKELEY, CAL.
J. J. R. MACLEOD, M.B. - - - TORONTO
ROY G. PEARCE, M.D. - - AKRON, OHIO
W. C. MACCARTY, M.D. - ROCHESTER, MINN.
GERALD B. WEBB, M.D. - COLORADO SPRINGS
WARREN T. VAUGHAN, M.D. - RICHMOND, VA.
VICTOR C. MYERS, PH.D. - - NEW YORK

Contents of this Journal Copyright, 1921, by The C. V. Mosby Company—All Rights Reserved

Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIALS

The Chemical Control of the Respiratory Center

THE widespread muscular cooperation necessary for the proper performance of the acts of inspiration and expiration depends on the presence, somewhere in the brain, of a center dominating the activities of the lower spinal neurones that innervate the respiratory muscles. In order that this chief center may bring about increase or decrease in respiration according to the needs of the body, it must, as it were, be kept informed of the respiratory requirements. This information might be supplied either through the peripheral nervous system by afferent impulses, or through the blood by chemical messengers or hormones. Although both of these methods of control are used the more important is believed to be the latter. It is also believed that this hormone is related to the changes in the gases of the blood.

The two gases concerned are oxygen and carbon dioxide, each of which exists in the blood partly in a combined and partly in an uncombined or free state, and it is natural to assume that it must be the latter portion that affects the activity of the respiratory center. This free portion creates a partial pressure, or tension, of the CO_2 or O_2 in the blood. On a first examination,

there is nothing to indicate which of the two free gases in the blood is likely to be the respiratory hormone, and it is accordingly necessary, in approaching the subject, to investigate the effect on respiration of each gas separately. Let us therefore consider the effect of increasing the free carbon dioxide of the blood on the activity of the respiratory center. This can be done by causing animals to inspire air containing varying percentages of carbon dioxide. The most definite results of this type were obtained some years ago by R. W. Scott[1] who, to avoid errors incurred by using anesthetics, employed decerebrate cats for his experiments. Such animals breathe normally for considerable periods of time and are, of course, entirely insensible to pain because of the removal of the higher centers. They react to increasing percentages in inspired air in a remarkably precise manner. The first increase in respiration is observed when the percentage of carbon dioxide in inspired air has risen to a little above one, and then, as the percentage is further raised, the respiratory volume increases in direct proportion, so that when about 5 or 6 per cent of carbon dioxide is being inspired it is 4 or 5 times greater than the normal. Without further investigation one could not of course say from this experiment that the increased breathing had been due to an increased percentage of free carbon dioxide in the blood supplying the respiratory center. It might, for example, be due to irritation of afferent nerve fibers in the respiratory passages. Direct analysis of the blood in Scott's experiment, however, showed that the free carbon dioxide was increased in proportion to the increase in breathing. Having established this fact the next question to present itself was: does this free carbon dioxide in the blood act directly on the center or indirectly, in that it brings about a change in some other property of the blood? The property to be thought of in this connection is the hydrogen-ion concentration, which, it will be remembered, is an expression of the reaction of the blood. An increased amount of free carbon dioxide would be expected to raise the hydrogen-ion concentration, because of its acidic qualities. It was accordingly necessary to see whether increased acidity of the blood actually occurred and, by the use of the colorimetric method, Scott showed that it did. We are therefore face to face with the problem: is it the free carbon dioxide as such, or the accompanying increase in hydrogen-ion concentration that is the real stimulus of the respiratory center? Evidence in favor of its being a change in hydrogen-ion concentration seems to be afforded by the observation that injection of acid intravenously does cause an increase in respiration and to a lesser degree, injection of alkali decreases it. It should be pointed out here, however—and this fact is often disregarded—that although intravenous injection of other acids than carbonic does cause hyperpnea, the results are much less definite and quantitative and, moreover, the injections are much more likely to cause serious damage, resulting very frequently in the death of the animal. We have observed, for example, that a sufficient injection of hydrochloric acid to bring about the same effect that is produced by inspiration of about 5 per cent of CO_2 is invariably followed by the death of the animal. In both cases, that is both with CO_2 and with mineral acid, the hydrogen-ion con-

centration of the blood is increased and the respirations are stimulated, but in the one case, namely with fixed acid, other effects are produced which are likely to have a fatal issue. Similar differences in the relative effects of carbonic acid and other acids on the respiratory center have been demonstrated by Hooker and others, who in perfused preparations of the respiratory center showed that solutions containing free carbon dioxide had a much greater stimulating influence on the center than other solutions having the same hydrogen-ion concentration. These experiments suggest that the free carbonic acid in the perfusion fluid must have a specific action of its own in stimulating the respiratory center and the final proof that this is so, is afforded by further experiments by R. W. Scott.[2] Quantities of alkali sufficient to produce a decided diminution in hydrogen-ion concentration of the blood, that is to say, a true alkalosis, were first of all injected into decerebrate cats. Varying percentages of carbon dioxide were then administered to these animals and it was found that the increase in breathing thus induced was very much the same as in normal animals; thus, the breathing became increased several times at percentages of 5 or 6 carbon dioxide in the inspired air although the blood *was still decidedly on the alkaline side.* In other words, there was marked stimulation of the respiratory center with the blood more alkaline than normal, but containing an excess of carbonic acid. Free CO_2 in the blood must therefore have an effect on the respiratory center that is independent of the change it can bring about in the hydrogen-ion concentration of the blood. It is not, of course, to be denied that change in hydrogen-ion concentration may affect the center—the experiment already referred to in which mineral acids were injected intravenously shows conclusively that this is the case—but the point is that *free carbon dioxide, besides affecting the hydrogen-ion concentration, can also specifically stimulate the respiratory center.*

Now the question is: how does carbon dioxide do this? If we can answer this question in the case of the respiratory center, certain other physiologic effects of free carbon dioxide that are apparently independent of the change in hydrogen-ion concentration will be elucidated; for example, its effect in stimulating the movements of the isolated intestine and its effect on the heart beat. The answer to the question has recently been furnished by some most interesting experiments performed by Jacobs[3] of the University of Pennsylvania. By observing the behavior of certain ciliated and flagellated protozoa in solutions in which the same hydrogen-ion concentration was secured by using different acids, including carbonic acid, it was found that the reactions towards the solution containing carbonic acid were quite different from those of other acids. The main difference was that the solutions containing carbon dioxide produced much more marked changes within the cell than other acids which on the other hand acted much more on the cilia than carbonic acid. Similar experiments with tadpoles confirmed the conclusions that the carbonic acid must penetrate the cell wall much more readily than other acids do. In short then, the specific action of carbon dioxide must be related to its ready penetration of the cell membrane and the possibility has to be considered that after its penetration it develops its effect by produc-

ing within the cell a local increase in hydrogen-ion concentration. According to this interpretation, it penetrates the cell membrane as a molecule of CO_2 or H_2CO_3 and in the protoplasm reacts with other inorganic salts to produce free hydrogen-ions. Further evidence of the great penetrating power of the carbonic acid molecule is afforded by the fact that solutions containing free carbon dioxide, even though they are slightly alkaline towards indicators, may taste slightly acid. More recent experiments by Jacobs[4] throw further light on this interesting property of carbon dioxide. He has found, for example, that the petals of certain flowers which are normally of a bluish tint become decidedly pink when they are bathed in a solution of sodium bicarbonate containing some free CO_2 but not enough to give it an acid reaction. The change to pink indicates that the interior of the petal has acquired an acid reaction although the outside fluid in which it is bathed is slightly alkaline. He has also constructed an interesting model demonstrating the same principle. This consists of a small bottle nearly filled with a faintly alkaline solution containing some indicator, on the top of which is a layer of xylene, the mouth of the bottle being closed by a thin membrane of celloidin. By immersing this bottle in a fluid composed of weak sodium bicarbonate containing a sufficient amount of free carbon dioxide to give $p_H > 7.4$ (therefore alkaline in reaction) it is found that acid penetrates through the xylene into the neutral solution in the bottle which it causes to become acid in reaction. The explanation is that the xylene has a specific solubility toward carbon dioxide which it therefore transfers to the neutral solution where, by chemical action with the salts in this solution free hydrogen-ion is produced.

These experiments then, show clearly that whereas the respiratory center is sensitive towards changes in hydrogen-ion concentration however produced, it is still more sensitive to changes in free carbon dioxide because through its great penetrating power this enters the cells of the respiratory center and causes local changes in H-ion.

These fundamental experiments indicate the importance of measuring the tension of carbon dioxide in the blood supplying the respiratory center in order to study the conditions of its hormone control. The method of determining the tension of a gas in such a solution as blood, is to expose the solution to a confined atmosphere which is then analyzed for the percentage of the particular gas in question. From the law which states that the percentage of gas in simple solution in a fluid will be proportional to the percentage of that gas in the atmosphere, it follows that this must be proportional to the tension. In the animal body the conditions for this measurement are fulfilled by the relationship into which the blood comes with the air in the alveoli of the lung. The percentage of carbon dioxide in the alveolar air must, in other words, be proportional to the percentage of carbon dioxide in simple solution in the blood plasma as it leaves the lung. It is for this reason that analysis of alveolar air for carbon dioxide has come to occupy so prominent a place in physiologic and clinical investigation.

From what has been said, it might be expected that in all cases where breathing has increased, there should be an increased percentage of carbon

dioxide in the alveolar air. Actual examination, however, shows that this is very seldom the case, it being usually found that in cases of hyperpnea the percentage of carbon dioxide in the alveolar air is sub-normal. How then does this fit in with our hypothesis? To explain it we must adduce that these cases of hyperpnea are dependent on stimulation of the respiratory center by other factors than free carbon dioxide and the question arises, what may these be? Several possibilities present themselves, one, that other acids than carbon dioxide have appeared in the blood, such as is known to occur in the late stages of diabetes and possible also in nephritis; second, that stimulation has occurred as a result of action by other respiratory hormones such as deficiency of oxygen in the blood, and third, that the respiratory center itself has become stimulated by afferent nervous impulses. It would take us beyond the scope of this review to consider each of these three possibilities in detail and we must confine ourselves mainly to the second, namely, that the center is stimulated by the decrease in the free oxygen of the blood.

That decrease in oxygen in the inspired air can stimulate the respiratory center is readily demonstrated by experiments both on laboratory animals and on man. Oxygen deficiency in the inspired air occurs in the case of man, for example, during life at high altitudes, in aeroplaning and sometimes in mining. All candidates for the aviation service in this and other countries were tested as to their reaction toward a gradual decrease in oxygen percentage in the inspired air and it was always found during these tests that when this percentage had been lowered to somewhere about 14 the respirations became excited. Deficiency of free oxygen in the blood plasma might also, however, be brought about in ways independent of a decrease of oxygen in the inspired air. For example, a diminished available amount of hemoglobin to take up this gas, as occurs, for example, in carbon-monoxide poisoning, or a decrease in the movement of blood through the pulmonary capillaries, as occurs in decompensated heart disease, will so reduce the O_2-carrying power that the tension soon falls in the capillaries. Barcroft[5] has proposed terms for the various forms of anoxemia (as general condition of oxygen deficiency in the blood is called) namely, anoxic when it is due to deficiency of oxygen in the inspired air; anemic, when due to faulty oxygen-carrying powers of the blood; and stagnant, when due to faulty circulation through the tissues. However produced, the anoxemia besides stimulating respiration produces many other effects, many of which are attributable to faulty oxygenation of nerve centers—numbness or tingling of the fingers, anesthesia, changes in visual acuity and in hearing, loss of memory, inability to think, muscular tremors, are for example frequent symptoms, especially in cases where the oxygen percentage has been lowered rapidly.

One effect of the oxygen deficiency on the respiratory center is that it causes this to act very rapidly, producing shallow, rapid breathing. This leads to an aggravation of the anoxemic condition because by its shallowness each breath does not cause so adequate a ventilation of the pulmonary alveoli. The anoxemic condition becomes, therefore, rapidly worse and as Haldane, Meakins and Priestley[6] have shown a vicious circle becomes established.

As a result of the increased breathing, brought about by anoxemia or in any of the other ways mentioned, the blood becomes overventilated in the alveoli with the result that the free carbon dioxide becomes excessively removed from it. It is "blown off" or "washed out" of the blood and as a result of this reduction in free acid the blood tends to become more alkaline in reaction, in other words, a condition of *alkalosis* becomes established. To compensate for this excess of alkali, there comes to be an increased excretion of alkali by the urine, which can readily be detected by titration with phenolphthalein, and at the same time the excretion of ammonia becomes markedly decreased indicating that to keep down the alkali the body is now converting as much ammonia as possible into urea, which is neutral. But the combination is not perfect, with the result that for some time the blood is decidedly alkaline in character. If the alveolar air be examined in these cases the percentage of carbon dioxide will be found below the normal. A similar condition of alkalosis may also result from hyperpnea due to any cause, either voluntarily, or because of pain, or because of incomplete anesthesia in surgical operations. It is the condition called "Acapnia" by Y. Henderson. After the hyperpnea has ceased, the alkalosis will tend to be compensated by lessened breathing, to retain CO_2, with a low alveolar—CO_2.

BIBLIOGRAPHY

[1]Scott, R. W.: Am. Jour. Physiol., 1917, xliv, 196.
[2]Ibid., 1918, xlvii, 43.
[3]Jacobs, M. H.: Ibid., 1920, li, 321.
[4]Ibid., 1920, liii, 457.
[5]Barcroft, J.: The Lancet, London, 1920, cxcix, 485.
[6]Haldane Meakins and Priestley: Jour. of Physiol., 1918, lii, 420.

—*J. J. R. M.*